ANOMALOUS CHROMOSOME PAIRING IN THE MALE DROSOPHILA PSEUDO-OBSCURA

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INTRODUCTION

The chromosome complement

The Diptera have not proved to be the most favorable material for the study of chromosome behavior, but the extensive work of STEVENS, METZ, KEUNEKE, and others has shown two exceptional properties in most sections of the order, namely, the pronounced somatic pairing of homologous chromosomes at mitosis, and the apparent lack in the male of the characteristic stages of pachytene pairing and chiasma-formation found at meiosis in nearly all other organisms. Further, GUYÉNOT and NAVILLE have indicated that meiosis is probably normal in the female of Drosophila and Calliphora, thereby showing a difference that may be correlated with the difference in regard to crossing over between the two sexes.

In order to analyze the peculiar character of pairing in the male it seems best to concentrate on those critical stages from diakinesis to anaphase of the first meiotic division when the structure of small paired chromosomes and the mechanics of their changing relationships can alone be accurately described. The object of the present study is to examine the form of the paired chromosomes in the light of those general principles of chromosome mechanics which I have earlier attempted to define, and thus to find out whether these principles may be applied to Drosophila. The processes of inference are therefore necessarily more elaborate than have been attempted hitherto in this kind of work.

For this purpose I have been fortunate in being able to use the admirable preparations of *Drosophila pseudo-obscura* made by Professor TH. DOBZHANSKY in the course of his studies of races and inter-racial hybrids of this species. Various methods have been used by DOBZHANSKY, the most suitable for my observations being fixation in Benda's solution and staining with haematoxylin. All the sections were cut at a thickness of 7μ. *Drosophila pseudo-obscura* Frol. is the North American species referred to as *D. obscura* by LANCEFIELD (1929), KOLLER (1932 a and b) and others, but it has been shown by FROLOWA and ASTAUROW (1929) to be distinct in morphology and in chromosome complement from the European species of this name. *D. pseudo-obscura* has three large pairs of autosomes with ter-

minal spindle attachments and a fourth which is small and spherical: this last corresponds morphologically with the fourth of *D. melanogaster* and is not usually visible at meiosis. The X chromosome has a median spindle attachment and each of its arms is longer than any autosome. DOBZHANSKY (unpublished) has shown that there are four racial types which differ in the form of the Y chromosome (figures 1 and 2). He has also shown

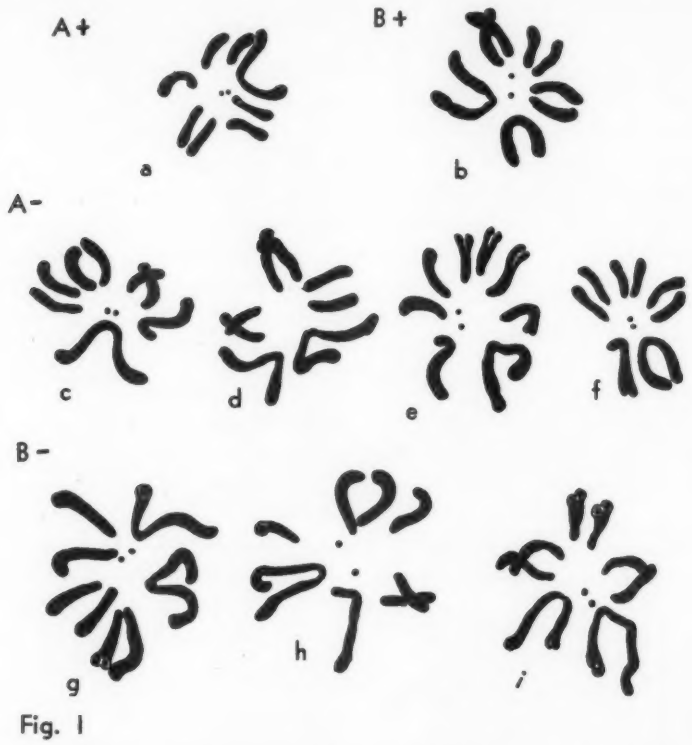


Fig. 1

FIGURE 1.—Metaphases of spermatogonial divisions of the four racial groups of *Drosophila pseudo-obscura*. Strong A, a (Texas); Strong B, b (Seattle 4); Weak A, c (Seattle 1), d (La Grande 4), e (Pointed), f (Seattle 5); Weak B, g (Seattle 2), h (La Grande 2), j (Seattle 6). (Drawings lent by Prof. DOBZHANSKY) ×6200.

that they differ in the kinds of hybrids they produce *inter se*, both in morphology and in the behavior of the chromosomes at meiosis (DOBZHANSKY and BOCHE 1933). I have been able to study meiosis in all the four pure types and their comparison has been of critical value in analyzing the pairing of X and Y.

THE PAIRING OF THE AUTOSOMES

Of the autosomes the three large pairs are alone regularly visible at meiosis. During diakinesis they assume a definite outline. They are rod

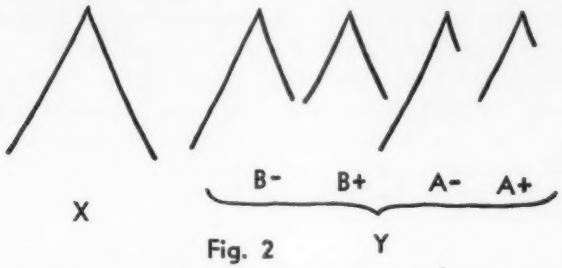
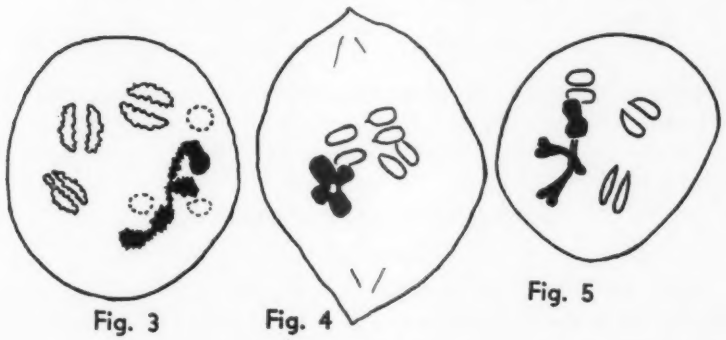
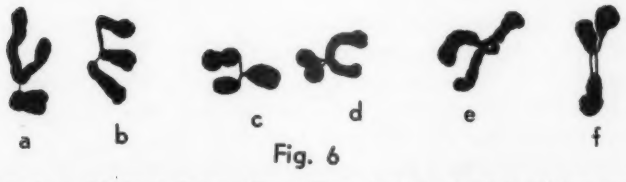


FIGURE 2.—Diagram to show the relation of the Y, in the four racial groups, to the X chromosome, which is constant.

shaped and their proximal and distal ends are not distinguishable. The bivalents appear from the side to be double, consisting of two clearly separated rods (figures 3–6). In end view, however, they are seen to be quadri-





FIGURES 3–6.—Diakinesis. Figure 3, Texas; Figure 4 and 5, Seattle 5 with long and short arm association of Y; figure 6, X–Y configuration, a–d in Seattle 5, e in Pointed, f in Texas. (In these and succeeding camera lucida drawings of the first division the sex chromosomes are shown solid, the autosomes in outline). ×6200.

partite and the four parallel rods (the chromatids) composing them are equidistant or nearly so (figure 36). The relative positions of the ends of the chromosomes indicate that at this stage the spindle attachments, which are invisible, are not turned away from one another as they are at metaphase. When the spindle develops, the attachment ends of the partners are turned towards opposite poles so that the chromosomes are oriented for regular segregation in the axis of the spindle (figure 9 and following). Anaphase separation, however, often begins before a flat plate is formed (see METZ 1926, on several Drosophila species). At anaphase the sister chromatids, held close together, are gradually drawn away from their partners at the spindle attachments, the length of parallel association de-

Fig. 7 Fig. 8

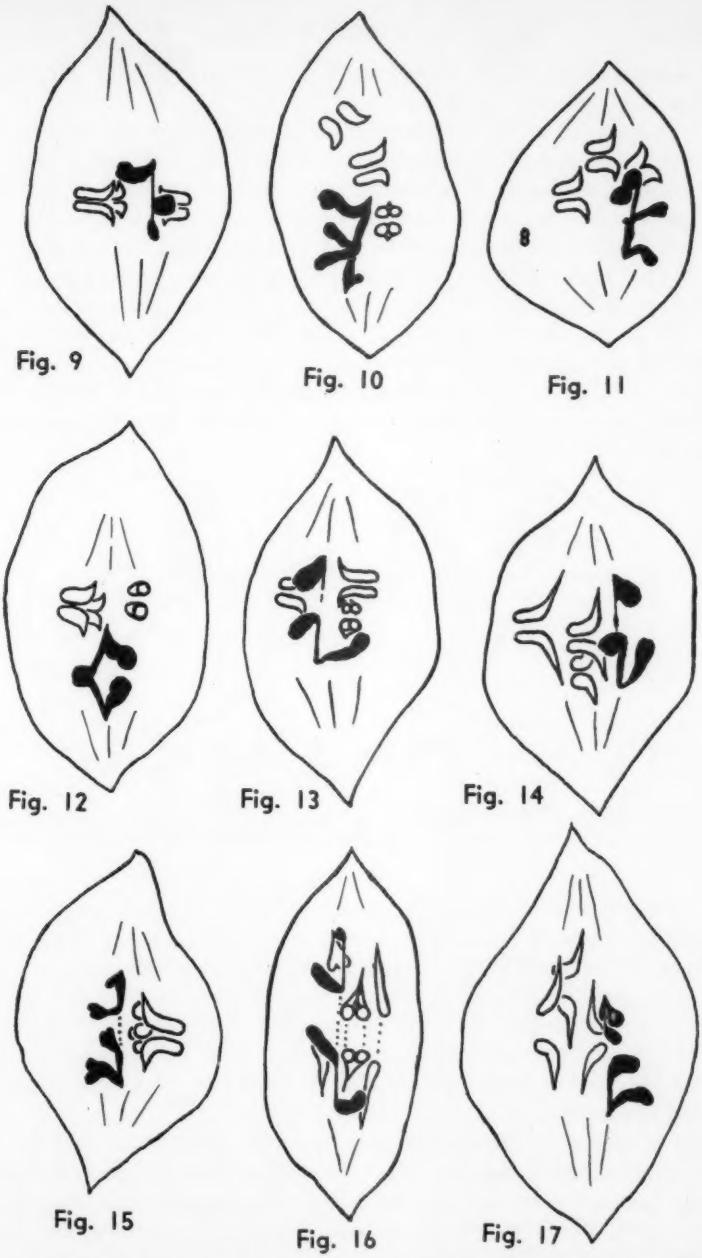
FIGURES 7 AND 8.—Polar views of first metaphase in Texas and Seattle, 5, ×6200.

creasing as the spindle attachments move further apart (figure 14 and following). In their behavior during interphase and second division the autosomes, as also the sex chromosomes, are entirely normal (figure 31 and following).

THE PAIRING OF THE SEX CHROMOSOMES

Normal behavior

METZ (1926) and STEVENS (1908) have shown that in various dipteran species, the X and Y chromosomes pair in a short intercalary portion of their length to give a cross-shaped configuration at diakinesis and metaphase. The present observations agree in showing that the X and the different kinds of Y are always associated in the neighborhood of the spindle attachment of the two chromosomes and for a very short length. But the configurations at diakinesis seem to be of two kinds: those showing a close connection between the two chromosomes and those showing a clear space between them. The first kind sometimes appears as a cross with a loop in the middle (figure 4). This difference will be considered in the light of the metaphase observations, which alone give critical results; for with the autosomes, as also with the sex chromosomes, the spindle attachments do not reveal themselves at diakinesis by their mutual repulsion as they do



FIGURES 9–17.—First metaphase and beginning of anaphase in side view. Figure 9, Texas (Strong A); figures 10–16, Seattle 5 (Weak A); figure 17, Seattle 6 (Weak B). Figures 9, 11, 12, 13, 14, and 17 show short arm associations, the rest, long arm. ×6200.

in other organisms; they therefore cannot be placed in relation to the point of pairing until metaphase.

The most important for the study of metaphase and anaphase are the weak-A races with the greatest contrast in size between the two arms of the Y chromosome (figure 2). In these races two kinds of association can be distinguished in side view of every complete nucleus. The first kind is that in which the Y is associated with the X in its long arm (figures 10 and 20 a, b, c). The unattached short arm is then lying towards the pole and the long arm is lying in the middle of the figure, balancing the associated arm of the X. The second kind is that in which the short arm of the Y is associated with the X and the long arm is lying free towards the pole (figures 11–14 and 20 *d–j*). This second kind probably corresponds with the diakinesis figures in which no connection between the two chromosomes is visible.

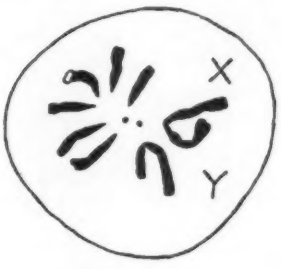


Fig. 18

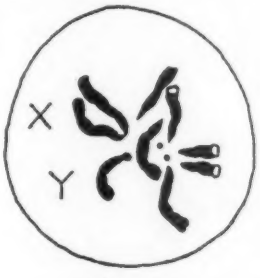


Fig. 19

FIGURES 18 AND 19.—Spermatogonial metaphases in Seattle 2 and Texas to show the difference in lengths of the two arms of the Y chromosome; this is important in interpreting figure 20. ×6200.

At anaphase in the weak-A races the two kinds of association give characteristic types of separation (figures 16 and 17). In the other races (strong-A and weak-B) the contrast between the two arms of the Y is not so great and the distinction between the two types is more difficult, although it has been attempted. It then appears that, while the weak-A and strong-A races have an equal number of short-arm and long-arm associations, the weak-B races have more in the short arm (21 to 8 in Seattle 6, 8 to 5 in Seattle 2).

As anaphase begins, the portion of each chromosome between its spindle attachment and the point of association is drawn into a fine thread and continues so for a short while after the separation has taken place (figure 16). The lengths of chromosome distal to the point of connection remain unaffected by the separation.

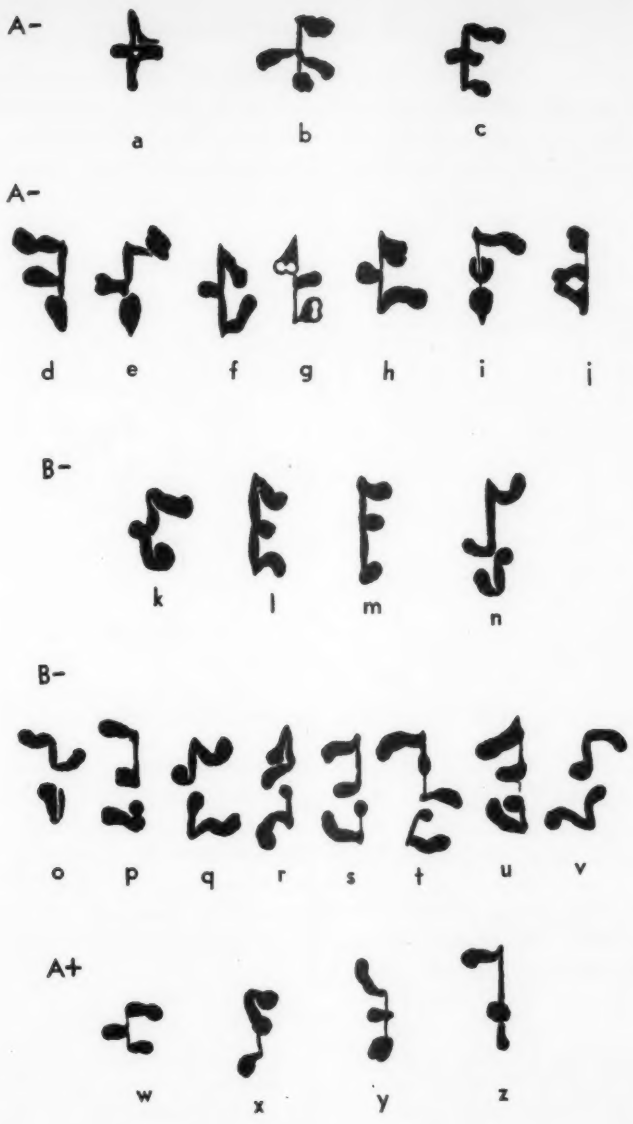


Fig. 20

FIGURE 20.—The XY bivalents to show the distinction between the two positions of association in weak and strong A, and in weak B races. The Y chromosome can only be distinguished at metaphase with long arm association. It is then placed below. a–c, long arm association; d–m, short arm association; n, long arm association; o–z, short arm association (o–v in anaphase, Y chromosome always distinguishable). a, b, e, h, i, j,—Seattle 5; c,—Pointed; d,—La Grande 2; f, g,—Seattle 1; k–r,—Seattle 2; s–b,—Seattle 6. × 6200.

Precocious separation

METZ (1926) has pointed out that the sex chromosomes, especially the Y, are condensed in advance of the autosomes during prophase in Drosophila. In their anaphase separation they also show occasional precocity in rela-

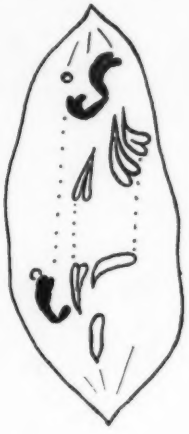


Fig. 21

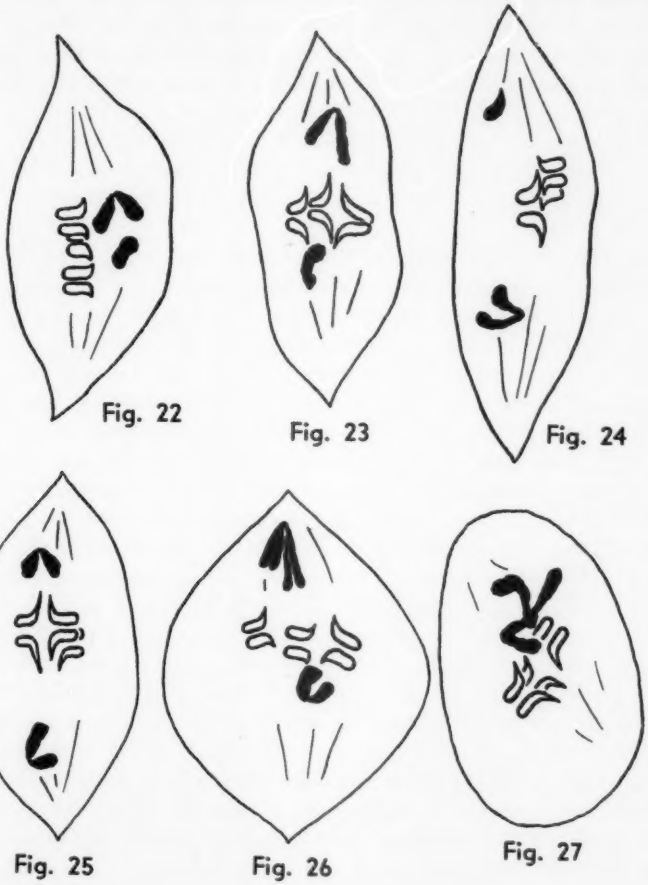
FIGURE 21.—Normal first anaphase in Seattle 5. ×6200.

tion to the autosomes (figures 22 to 26). In the stock Seattle 6 (weak-B) they separated precociously in eight divisions out of thirteen recorded; in Seattle 5 (weak-A) once in seven divisions. It sometimes happens, however, that the X-Y figure is behind the autosomes in orientation on the plate at metaphase (figure 27, Seattle 6). This has been found to be usual in *Drosophila melanogaster* by HUETTNER (1930). The irregularity is therefore perhaps best considered as an error in time coördination between the sex chromosomes and the rest.

Non-disjunction

Since BRIDGES' discovery of XXY females with both X's derived from the mother, the so-called "non-disjunction" of the sex chromosomes has been the subject of much genetic study. Females of the XXY constitution have been found in *D. willistoni*, *D. phalerata*, *D. simulans*, *and* (SCHULTZ and REDFIELD unpub.) *D. pseudo-obscura* itself. It is known also in *D. melanogaster* that the male can produce sperm with both X and Y or with neither sex chromosome, but their frequency is difficult to determine (STERN 1929).

Recent observations, particularly of plants, have made it clear that failure of pairing, not failure of separation, was the most likely cause of the abnormality since such failure is always followed by random distribution of the unpaired chromosomes. In *D. pseudo-obscura* I have found the X and Y unpaired at the first division in three nuclei and the results of



FIGURES 22–27.—Aberrant time coördination of sex chromosomes and autosomes in separation at first anaphase. Figures 22–26, precocity of sex chromosomes; figure 27, delay of sex chromosomes; figures 22 and 24, Texas; figure 23, Seattle 5; figures 25 to 27, Seattle 6. ×6200.

their passage to the same pole in five more (figure 28 and following). In the first divisions the X is lying near to one pole, while the Y lies on the

equator. In the second divisions both chromosomes have passed to the same pole.

THE MECHANICS OF PAIRING

The autosomes

I have described the pairing of the autosomes as anomalous because it differs from normal meiotic pairing in three respects: (1) the equal or almost equal association of chromatids in fours throughout their length at diakinesis and metaphase, (2) the sudden change in relationship of spindle attachments between diakinesis and metaphase from attraction or indifference to sharp repulsion; (3) lack of regular orientation on the metaphase plate. With these properties is perhaps associated a reduction of pachytene pairing, in regard to which, however, interpretation is inconclusive.

The first of these abnormalities—the equal association in fours—so far as it concerns diakinesis, may be considered to be, as METZ and KEUNEKE have suggested, merely the final stage of a somatic pairing which is progressively increased during the preceding spermatogonial divisions. In my own words (see DARLINGTON 1932, p. 342) I would say that a specific attraction (A_2) is manifested in somatic pairing. This always reaches an equilibrium with the non-specific repulsion (R_1), due to surface electrical charges, which is a universal property of all double chromatids during the dynamic stages of mitosis and meiosis. The somatic pairing attraction seen during spermatogonial mitoses increases at meiosis until it equals or nearly equals the one-to-one attraction between chromatids (A_1) so that the four chromatids come to be nearly equidistant. In normal meiosis the four chromatids fall apart into two pairs which repel one another and are held together only by the exchanges of partner, or chiasmata, between them. In the male Drosophila there is evidence of very slight repulsion (in equilibrium with attraction) but no evidence of chiasmata. This hypothesis would be tested by observation of meiosis in a tetraploid nucleus in which all eight chromatids of one type should be equally spaced. The variation in the distances apart of the four chromatids between diakinesis and metaphase (top of figure 36) is evidence of the different quality of the two kinds of attraction, the primary attraction within pairs (A_1) and the secondary attraction between pairs (A_2). Whatever interpretation be put upon the observations of the fixed and stained material, this comparison between successive stages must have some validity.

But when we apply this view to the anaphase separation we meet with a difficulty. Both in mitosis with somatic pairing and in meiosis in polyploid plants with "secondary pairing" we have the same attraction, A_2

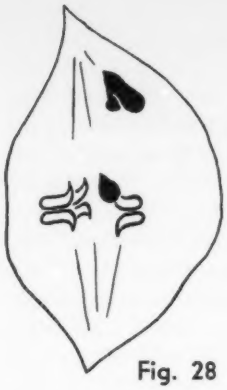


Fig. 28

Fig. 29

Fig. 30

a

b

Fig. 31

c

a

b

Fig. 32

a

b

Fig. 33

a

b

Fig. 34

FIGURES 28–33.—"Non-disjunction" of X and Y. Figures 28 (Texas) and 29 (Seattle 6), X lying towards one pole and Y on the plate at first metaphase; figure 30 (Seattle 4), X lagging at first telophase after having failed to pair; figure 31, a and b (Seattle 4) normal interphases, each with a Y chromosome, c (Seattle 5) interphase with X and Y; figure 32, a and b (Seattle 5) normal second metaphases, a, with Y, b, with X-fourth autosome visible; figure 33 a and b (Seattle 5), sister second metaphases resulting from "non-disjunction" of X and Y. ×6200.

FIGURE 34.—Normal second anaphases (Seattle 5); a with Y, b with X. ×6200.

(although in plants it does not manifest itself during prophase). *But this attraction never determines segregation* (see DARLINGTON 1932). Clearly at meiosis in Drosophila something else beyond A_2 is responsible for the regularity of segregation. The second anomaly indicates what this is.

At diakinesis the spindle attachments of the chromosomes are not visible, but the ends at which they lie are clearly not turned away from one another as they are at metaphase. I have suggested that the orientation of the metaphase chromosomes—determining separation at mitosis, segregation at meiosis and the fusion of chiasmata at the ends in terminalization—is dependent on the repulsion (R_2)[1] between the spindle attachments. These are double for each chromosome at mitosis as well as for each bivalent at meiosis so that the orientation in the two cases is the same. The occurrence of segregation therefore demands that the bivalent should have a double, and not a quadruple spindle attachment. The meiotic bivalent must therefore have a delay in the division of the spindle attachment relative to the development of the spindle, in fact a postponement to the second meiotic division. This precocity of the external changes relative to those internal to the chromosomes is, as I have pointed out, characteristic of meiosis.

Fig. 35

FIGURE 35.—Right, type of autosome bivalent illustrated by WOSSKRESSENSKY and SCHEREMETJEVA and by HUETTNER in *Drosophila melanogaster* with median spindle attachment. Left, type formed in *D. pseudo-obscura* with terminal spindle attachment, showing that the characteristic cross shape in *D. melanogaster* is misleading since it is not due to a chiasma.

The repulsion of the undivided spindle attachments accounts sufficiently for the separation of the chromosomes at first anaphase in Drosophila. But in normal meiosis repulsion sets in before metaphase, probably as a symptom of the external precocity, and gives rise to greater or less terminalization of chiasmata between diplotene and diakinesis. In Drosophila this repulsion does not appear until metaphase. If it did it would perhaps overcome the attraction A_2, and lead to premature disjunction during prophase. It therefore seems that reduced precocity is a second

[1] This is the repulsion which I hold to be responsible for BELAR'S "autonomous" separation (HUTH 1933).

characteristic of this abnormal meiosis, and this assumption is reasonable since it also accounts for the failure of prophase pairing and for the rather slight extra contraction of the chromosomes.

There are two points in this argument that require elaboration. My reason for assuming that the beginning of spindle attachment repulsion (R_2) during prophase is externally conditioned is that unless external conditions changed during prophase the spindle attachments should repel one another from the beginning of prophase since their repulsion effected the preceding anaphase separation. And further, only one internal change can be supposed to befall the spindle attachments during meiosis, namely, the splitting which leads to the separation of chromatids at second anaphase. I am supposing, therefore, that the spindle attachments potentially repel one another throughout the meiotic prophase, but that external conditions do not permit repulsion until diplotene in normal meiosis (leading to varying degrees of terminalization) and until metaphase in Drosophila. My reason for considering external and internal changes as two individual systems is merely for convenience of handling. Varying terminalization indicates that spindle attachment repulsions may vary independently of other external factors, but they need not be supposed to do so in the present instance.

I therefore conclude that the special behavior of the autosomes depends on the coördination of two special properties: (1) exaggerated somatic pairing, (2) reduced precocity with a consequent avoidance of pachytene pairing and of prophase repulsion of spindle attachments.

The third anomaly, the failure to form a metaphase plate, may be due either to a failure in time coördination between spindle and chromosomes, which will be referred to later, or to an exceptionally weak polar repulsion (R_3) such as I have assumed to be responsible for arrangement in a flat plate. The latter would be the opposite condition to that giving the "hollow spindle" where the strong polar repulsion forces all the chromosomes to the edge of the plate.

Note on the Classification of Forces. I have in the past looked upon the primary attraction A_1 as different in cause as well as in effect from the secondary attraction, A_2, for this reason: A_1 leads apparently to the most intimate association analogous to the contact of solid bodies, while A_2 merely modifies the equilibrium position resulting from the operation of other forces. The present work on Drosophila, however, does not support or require this distinction. It is possible to consider both the attractions as determining an equilibrium position. It is then possible to suppose (and I am indebted to Dr. G. W. BEADLE and Dr. JACK SCHULTZ for this suggestion) that a common force of attraction exists between identical genes and that this attraction is almost or entirely saturated by the approxima-

tion (or association) of genes in pairs. Secondary attraction, A_2, is then a residual attraction and its variation is merely the variation of degree of saturation by pairing. In meiosis in the male Drosophila the saturation reaches its lowest level. Forces of repulsion due to surface charge (R_1) and localized charge at spindle attachment (R_2) and spindle poles (R_3) can likewise all be considered most conveniently as a unit in regard to physical causation. The distinction between them remains, however, convenient in description since their different distributions in space and time lead to their having different effects, and the analysis of these effects must precede the synthetic consideration of chromosome mechanics as a whole.

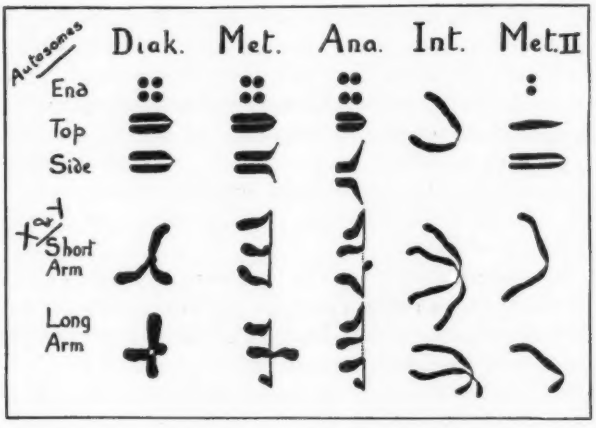


FIGURE 36.—Diagram showing the relationship between autosome and X–Y pairing in a weak A race of *D. pseudo-obscura* during successive stages of meiosis. The interphase column shows merely lateral views of the chromosomes and the last two stages of X and Y are, of course, indifferently derived from long and short-arm chiasma bivalents.

The sex chromosomes

The X and Y chromosomes have an entirely different relationship at the first metaphase, as shown by both structure and behavior, from that subsisting between autosomes. Thus they are only in contact over a short portion of their length and this is evidently not the whole of the homologous segment. In this portion they come into closer contact than the autosomes, sometimes, and at other times rather less close—no visible connection joining them. Other parts, even though homologous, lie apart giving the cross figure clearly owing to their mutual repulsion (R_1). In the short paired region at diakinesis there is sometimes an open space visible between two points of contact. At metaphase a state of tension develops

between the spindle attachments and the point of association, and the chromosomes do not separate gradually at this point, but suddenly.

These observations lead to a common conclusion, namely that we are here dealing with a normal case of attraction between pairs of chromatids—not between fours—and that the four chromatids are held together by exchanges of partners between them, that is, chiasmata. Such chiasmata would need to be reciprocal, since both ends of X and Y are dissimilar, and close together as shown in the diagrams (figures 36 and 37, and DARLINGTON 1931, diagram III).

The exchange of partners, being inferred from general considerations, would seem to provide a purely formal explanation of the X-Y pairing in the absence of more specific evidence. Such evidence is provided by other peculiarities of behavior which can be accounted for in the same way. The occurrence of pairing on both sides of the spindle attachment shows not only that the homologous segments extend on both sides, but also that they may or may not be associated at metaphase. The characteristic chance formation of chiasmata on one side, or the other, of the spindle attachment demands this variation in association; A_2 attraction will not account for it. Then the sex chromosomes fail to pair in a proportion of cases, although the autosomes are regularly paired. This also can be accounted for by the chance failure of chiasma formation now known in so many species, particularly among the smaller members of the complement. Again the variation in appearance at diakinesis is accountable if we suppose that the homologous segment extends to the end of the short arm of the Y in weak-A races so that terminalization of the chiasmata in this arm will give a lateral chiasma with no visible connection while in the other arm a connection is always left, complete terminalization being impossible. Finally, the lack of time coördination between spindle, autosomes, and sex chromosomes found also in *D. melanogaster*, is an indication that two independent processes of development are at work in the same nucleus.

I therefore conclude that reciprocal chiasmata are formed between X and Y following normal pachytene pairing of the homologous segments and that these chiasmata are either in the short arm or in the long arm of the Y or—where metaphase pairing fails—in neither.

THE DISTRIBUTION OF ABNORMAL MEIOSIS

The general type of meiosis in animals, plants, and Protista is that in which the chromosomes repel one another at metaphase and are only held together by chiasmata formed during the pachytene pairing. (My application of the term "pairing" to prophase, as well as to metaphase, association has been found ambiguous. But the term synapsis, which I avoid, is even more so. It is used to refer to [1] the act of pairing, [2] the

condition of being paired, [3] the period during which pairing occurred, [4] the period when pairing was complete. To use an abstruse expression with an indefinite meaning seems to be an unnecessary offence.) The abnormality described here in regard to the pairing of X and Y chromosomes is relatively slight and is paralleled in other groups. The abnormality in the pairing of the autosomes is profound. How widespread is it? In the first place it is evidently characteristic of Drosophila. In *D. melanogaster*, STEVENS (1908), WOSSKRESSENSKY, SCHEREMETJEVA and HUETTNER have illustrated autosome bivalents which are quadripartite in side view and correspond with the bipartite bivalents of *D. pseudo-obscura* since the autosomes have median spindle attachments in the one species and terminal in the other (figure 35). The Russian authors describe these as "characteristic tetrads." This they are not for their four parts could not be four chromatids, in view of the position of the spindle attachment, but must be a pair of two-armed chromatids. Further, STEVENS' illustrations show the same simple parallel association in Eristalis, Sarcophaga, and Calliphora, and from KEUNEKE's observations the same method of pairing may be inferred. In these and in some of METZ's work the side views of first metaphase, which are so critical in analyzing bivalent structure, are not illustrated, but the detailed studies of successive stages leave no doubt that an abnormal and probably similar course of development is followed.

The following summary (based on the classification of IMMS 1925) includes the most convincing accounts and indicates the extent of the survey of meiosis in male Diptera.

Orthorrhapha

Series I. Nemocera: Culicidae—Culex, Anopheles, STEVENS 1911.
Choaborus, FROLOWA 1929.
Sciaridae—Sciara, METZ et al. 1926.

Series II. Brachycera: Asilidae—Asilus, Dasyllis, METZ et al 1922.

Cyclorrhapha

Series III. Athericera:

Aschiza,
Syrphidae—Eristalis, STEVENS 1908.

Schizophora-Acalyptratae,
Scatophagidae—Scatophaga, STEVENS 1908, KEUNEKE 1924.
Sciomyzidae—Tetanocera, STEVENS 1908.
Trypetridae—Tephritis, KEUNEKE 1924.
Ortalidae—Camptoneura, METZ 1916.
Drosophilidae—Drosophila (see text).

Schizophora-Calyptratae
Anthomyidae—Phorbia, STEVENS 1908.

Muscidae—Calliphora, NAVILLE 1932.
Lucilia, STEVENS 1908, KEUNEKE 1924.
Sarcophaga, STEVENS 1908, METZ 1916.

Series IV. Pupipara: (none).

The forms mentioned in this list may be divided into three groups according to the type of meiosis in the male. First, we find in the Culicidae normal types of bivalent chromosomes with terminal and interstitial chiasmata. Secondly, we find in the Sciaridae an abnormal meiosis without any chromosome pairing. Thirdly, we find all the other flies, the more highly specialized Brachycera and Athericera (which fall into one group according to the segmentation of the antenna), agree in having the Drosophila type of meiosis, without chiasmata except in the sex chromosomes.

It may now be said that there are four groups of abnormalities found at meiosis in the male:—Sciara, Drosophila, haploid-male, and coccid, this last showing an evolutionary series of changes from moderate to extreme types (SCHRADER 1931). Since, amongst other reasons, the female is probably normal in all these groups, we must suppose that the various special processes have arisen in the heterozygous sex from the normal meiosis. In the Drosophila group the sharpness of the abnormality, and the clearness and extent of its distribution, indicate that it arose from chiasma pairing at a remote period, while the difficulty of supposing an intermediate condition between chiasma pairing and secondary pairing indicates that it arose at a single step.

THE CYTOLOGICAL THEORY OF CROSSING OVER

The simplest hypothesis to relate the genetically inferred occurrence of crossing over with cytological observation is the simplified chiasmatype hypothesis of JANSSENS (DARLINGTON 1930). This supposes that all paired chromatids are sister chromatids so that (1) all crossing over between pairs of chromatids of partner chromosomes gives rise to an exchange of partners, or formation of a chiasma, between them and (2) chiasmata can arise in no other way. This hypothesis is supported by three kinds of observation; first, the observation that in a series of critical cases the cytologically demonstrable formation of new chromatid combinations, such as can only result from crossing over, accompanies the formation of a chiasma. These cases consist in special types of quadrivalent and trivalent in polyploids, of the figure-of-eight configuration in interchange heterozygotes and of special configurations in inversion heterozygotes and with interlocked chromosomes. The assumptions on which they depend for

validity are physiological, developmental, and morphological in the different cases, but naturally require in each case the additional assumption of chromosome continuity which is inherent in the statement of the problem (DARLINGTON 1932); secondly, the observation that the frequency of chiasmata agrees with the frequency of crossing over on this hypothesis, either in a particular segment of a chromosome (BEADLE 1932) or in the complement as a whole (DARLINGTON 1933); finally, the observation that the chiasmata show all the special properties that have been inferred in regard to crossing over, for example, interference, as shown by their numerical distribution (HALDANE 1931), variation in frequency and distribution subject to genetic and environmental conditions, a special relationship with the spindle attachment as shown by localization (DARLINGTON 1932), and a reduction in the heterozygous sex where crossing over is less frequent (CREW and KOLLER 1933, BRYDEN 1933). Chiasmata also show other properties which have not yet been discovered in regard to crossing over, such as indirect length-frequency relationships and this is owing, no doubt, to the restricted number of organisms available for comprehensive genetic study of the problem, and the uneven distribution of genes on chromosomes.

There has been one outstanding objection to this theory, namely that no difference was known between males and females in regard to chiasma formation such as exists in the complete suppression of crossing over in male Drosophila. This objection became particularly serious when it was found that chiasma formation, and therefore crossing over, was the general condition of metaphase pairing. Since critical information was generally lacking in regard to the female, and, in Drosophila, in regard to the male as well, the field was open for numerous speculations. There were two possible explanations of the behavior of the male on the chiasmatype hypothesis. One was that, as the SCHRADERS have found in the coccids, the female was normal—the chromosomes pairing by chiasmata presumably—while in the male, pairing was wholly abnormal, being determined by forces not operative or effective in the female. The other was that chiasmata were formed in the male as well as in the female, but occurred in inert regions and in such a way as to give no visible crossing over. If, as seemed most likely in the sex chromosomes, the region was intercalary, then the chiasmata must be formed by reciprocal crossing over. Consideration chiefly of the evidence of non-disjunction of the sex chromosomes and of the occurrence of the XY change led me (1931) to adopt the second hypothesis which was rendered more plausible by the discovery that the proximal third of the X is an inert region. It now appears that this assumption actually applies to the sex chromosomes, while the first assumption applies to the autosomes.

While, in the male, chiasma formation is suppressed or regulated so as to give no visible crossing over, in the female presumably one or more chiasmata are formed in each pair of chromosomes following and paralleling the genetically observed crossing over. Thus each chromosome will have a minimum of 50 percent crossing over in its total length if it pairs by chiasmata. Observation of the female has so far merely shown the presence of the normal pachytene stages which are absent or abortive in the male (METZ and others 1926, GUYÉNOT and NAVILLE 1929, HUETTNER 1930, NAVILLE 1932).

It was possible earlier in the absence of any cytological evidence, to suppose that chiasmata were formed equally in male and female and that their breakage was, as JANSSENS suggested together with his other hypotheses, responsible for crossing over. Then, by all the chiasmata being equational (DARLINGTON 1929), or by the failure of breakage (SAX 1930), the lack of crossing over in one sex could be explained. Thus would Drosophila have been saved for the chiasma theory of metaphase pairing. But since there are no chiasmata, in the male autosomes these *ad hoc* assumptions are left without theoretical use, as well as without observational evidence, while the requirements of the simple chiasmatype hypothesis are met in every respect.

The flies and rodents show two ways in which differences may arise between the sexes in regard to crossing over. The grasshoppers show yet a third. In these we find various conditions in the male—suppression of crossing over, a reduction of crossing over, or even between particular genes in some species, an increase (NABOURS 1929). This complicated situation becomes intelligible in terms of the chiasmatype hypothesis when we recall that the mapping of the chromosomes is still fragmentary and that in the Tettigidae in question not more than one chiasma is usually formed in each bivalent. If then this chiasma is always or usually formed in a particular region of the chromosome, the occurrence or non-occurrence of crossing over will depend on the position of the known genes in relation to this region. Different types of localization of chiasmata will then account for differences in crossing over frequency. Localization is difficult to identify in the Tettigidae which have a high degree of terminalization, but in the Acrididae it is well known to distinguish the behavior of different genera. In this regard it is important to notice that NABOURS and his collaborators now find (1933) that crossing over, which does not occur between particular genes in Paratettix does occur between their presumed homologues in female Apotettix and, even more frequently, in the same sex in Acrydium (individual experiments being here significant even though different experiments may be inconsistent). Differences between related species in regard to chiasmata and crossing over are therefore of the same kind as

those between the two sexes. The position now is that the simple chiasma-type hypothesis, like other hypotheses, cannot be proved. It can only be subjected to the possibility of disproof, by testing the many rigorous predictions that can be based on it. Like previous attempts the present study has failed to disprove it, and it seems that no difficulty now remains in applying it universally. Assuming its validity, we can begin to study crossing over cytologically, and chiasma formation genetically, thereby reducing the limitations of each method. I propose to discuss these limitations elsewhere.

TYPES OF DIFFERENTIATION OF SEX CHROMOSOMES

In so far as chromosome pairing is conditioned by crossing over, the type of crossing over in the sex heterozygote must condition the type of differentiation, that is, of development, by mutation and structural change, of non-homologous or "differential" segments in the pairing of sex chromosomes. We now have evidence of the existence of the three simplest crossing over or differentiation types which are, *a priori* to be expected, as follows. (The proximal segment is that which includes or adjoins the spindle attachment):

Type with distal homologous or pairing segments, proximal non-homologous or differential segments

Both ends may have pairing segments and then the differential segments are intercalary. Crossing over is unrestricted, but must be distal; the first division is therefore reductional for the differential segment. This type is analogous to the Oenothera and Rhoeo rings as I conceive them, and can give rise by interchange to the chains of four or five found in Humulus, as well as, by fragmentation of either X or Y, to the chain of three found in Rumex and Phragmatobia. This is the simplest type and it is found in all plants and in most animals with differential sex chromosomes (figure 37I).

Type with proximal pairing segments, distal differential segments

Crossing over is proximal and unrestricted, but if the pairing segment overlaps the spindle attachment, crossing over, occurring either on the near side of the inequality or on the far side, will give a reductional or equational division of the inequality according as 0, 1, or more chiasmata between the spindle attachment and the inequality maintain or break their chromatid relationship. This type is analogous to one kind of unequal chromosome in Phrynotettix and its occurrence may be inferred from genetic observations on *Bombyx mori* (GOLDSCHMIDT and KATSUKI 1931) and possibly from cytological observations of the mouse (CREW and KOLLER 1933, figure 37II).

Type with proximal intercalary pairing segments, distal differential segments in both arms

Crossing over is necessarily reciprocal. The requisite chiasma relationship is found in autosomes in *Fritillaria meleagris* and *Aggregata eberthi*. The sex chromosome type is known only in the genus Drosophila, but may be inferred in others of the Brachycera group. The pairing segments may be restricted to one arm, as in *D. melanogaster*, or extend to both arms, as in *D. pseudo-obscura* and no doubt in other species with both X and Y two-armed (figure 37III).

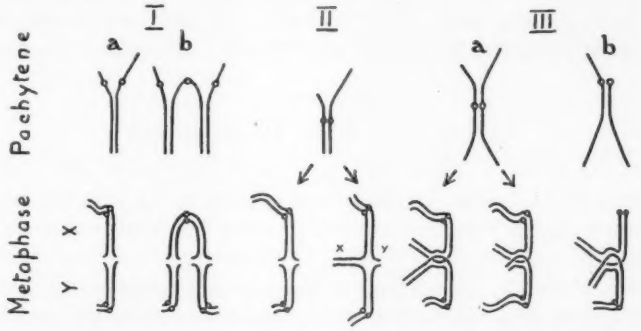


FIGURE 37.—Diagram showing the types of known or inferred relationship of X and Y and of chiasma formation and disjunction related with each. The spindle attachment is represented by a circle. At pachytene the homologous parts are those lying parallel, the X to the right except in Ib. Ia, the normal type in plants and animals with distal crossing over. Ib, *Rumex acetosa*, *Humulus japonicus*, *Phragmatobia fuliginosa*, with fragmented Y. II, type with spindle attachment in the homologous segments giving facultative equational first division, that is, with proximal crossing over (as in Bombyx). III, Drosophila type with intercalary pairing segment and reciprocal crossing over. *a*, *D. pseudo-obscura* with chiasmata in either arm. *b*, *D. melanogaster* with chiasmata in one arm.

This classification indicates that special mechanical properties of the pairing sex chromosomes have been the condition of various types of evolutionary change of which Drosophila provides a special example.

SUMMARY

1. The autosomes and sex chromosomes of *Drosophila pseudo-obscura* are in different ways anomalous in their method of pairing, as seen between diakinesis and first anaphase of meiosis in the male.

2. The autosome bivalents consist of four chromatids equally paired throughout their length without chiasmata, a condition made possible by exaggerated somatic pairing and changed precocity.

3. The sex chromosomes, as shown especially by their structure and behavior in races with an unequally armed Y, are paired by reciprocal

chiasmata either on one side or the other of the spindle attachment. Failure of pairing leading to "non-disjunction" was found eight times.

4. These two special mechanisms of pairing are compatible with the absence of crossing over in the male on the simple chiasmatype hypothesis. Reciprocal chiasmata between X and Y are a condition of a type of differentiation of the sex chromosomes in which the Drosophila group is unique. The special method of autosome pairing is probably characteristic of all the short-antenna section of the Diptera.

I am obliged to Dr. T. H. MORGAN for the facilities placed at my disposal during this study at the CALIFORNIA INSTITUTE OF TECHNOLOGY. I also have to thank Dr. TH. DOBZHANSKY, not only for the use of his preparations, but also for his experienced advice throughout the study.

APPENDIX I

The effect of the Gowen gene on the male

Since the X and Y chromosomes pair by a mechanism similar to that presumed in the female, Dr. BEADLE suggested to me that the effect of the Gowen gene, a third chromosome recessive which suppresses crossing over as well as pairing in the female, might affect the sex chromosomes in the male, although it is known not to affect the autosomes. I therefore crossed homozygous bobbed females by Gowen males. Normal disjunction of X and Y in these would give progeny with the bobbed character suppressed by either X or Y. Non-disjunction would produce XXY females and "extreme-bobbed" XO males (as shown by STERN). These might be of reduced viability, in which case the sex ratio would be affected. One cross (single male and female) yielded 130 males and 130 females. Others gave 8:5, 55:58 and 25:35. None of the males were bobbed, so that I conclude there is no evidence of non-disjunction of XY in the Gowen stock males.

APPENDIX II

Two recent studies bear on the interpretations and inferences made in the present account.

BAUER (Zeits. Zellforsch. u. mikr. Anat. **14**: 138–193) has described the normal meiosis of the nemoceran, *Tipula paludosa*, and has pointed out that in the males of the short-antenna Diptera, pachytene pairing is found in different degrees of degeneration and that this distinction corresponds with the systematic division of the Diptera. BAUER has thus arrived, on the evidence of pachytene pairing, at the same conclusion that I have reached on the basis of the correlated chiasma structure at metaphase.

GUYÉNOT and NAVILLE (Cellule **42**: 211–230) have studied oögenesis in female *Drosophila melanogaster*. Paired chromosomes, which correspond to

those seen at pachytene and diplotene in normal meiosis, pass directly into the diakinesis and metaphase bivalents. We must assume therefore (as I have assumed in the present study) that metaphase pairing in the female is conditioned by chiasmata, as in homozygous organisms generally. The authors consider however that the diakinesis and metaphase stages in the male correspond to those in the female. This I deny on two grounds: first, they are not observably derived from pachytene pairing and chiasma formation (as shown by GUYÉNOT and NAVILLE); secondly, their structure shows no evidence of such a derivation but on the other hand compels the assumption that different forces are responsible for the pairing (as shown in the present study, in contradiction to GUYÉNOT and NAVILLE). The issue therefore depends on the correctness of the one or the other interpretation of meiosis in the male.

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CALCULATING LINKAGE INTENSITIES FROM F_3 DATA*

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It has been shown by FISHER (1928) that no statistic for estimating linkage intensities can have a smaller sampling variance, in the theory of large samples, than that given by the solution of the equation of maximum likelihood. The method of maximum likelihood may be used, then, as a standard by which the efficiency of other formulae for calculating linkage intensities may be determined. FISHER (1930) determined the efficiency of several other methods for calculating linkage intensities from F_2 data. IMMER (1931) presented a study of the efficiency of the four-fold correlation method. FISHER and BALMUKAND (1928) provided a table to facilitate the calculation of linkages, from F_2 data, by the product ratio method. HUTCHINSON (1929) applied the method of maximum likelihood to problems of linkage between two genes when the characters were determined by duplicate of complementary factors, and IMMER (1930) provided tables which could be used to reduce to a minimum the labor necessary in calculating linkage intensities for various factorial combinations when the product ratio method is used.

It remained for FISHER (1930) to point the way to the solution of linkage problems when F_3 data are utilized in order to determine the F_2 genotypes. This can be accomplished most efficiently by an easy extension of the method of maximum likelihood. The present paper is a continuation of this study.

If we designate p (expressed as a decimal fraction) as the crossover percentage in repulsion crosses, with $1-p$ as the crossover percentage in coupling crosses, we may calculate easily the expected frequency of occurrence of the four expected phenotypes in F_2. The expected ratio for the *AB*, *Ab*, *aB* and *ab* phenotypes in F_2 will be $1/4\ (2+p^2,\ 1-p^2,\ 1-p^2,\ p^2)$. If we designate the observed frequency of occurrence of the *AB*, *Ab*, *aB* and *ab* phenotypes as a, b, c and d we may calculate the linkage intensity satisfying the equation of maximum likelihood from

$$\frac{a}{2+p^2}-\frac{b+c}{1-p^2}+\frac{d}{p^2}=0, \qquad \text{(I)}$$

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[1] The writer wishes to express his appreciation to Dr. R. A. FISHER for his criticisms and suggestions during the course of this study, begun while the writer was a Fellow of the NATIONAL RESEARCH COUNCIL at the ROTHAMSTED EXPERIMENTAL STATION, Harpenden, Herts., England.

or, expressed more conveniently for calculation, $n\ p^4-(a-2b-2c-d)\ p^2-2d=0$, where p or $1-p$ is the crossover percentage, as defined above, and n is the total frequency. The amount of information supplied by the data will be

$$\frac{2n(1+2p^2)}{(1-p^2)(2+p^2)} \tag{II}$$

and the standard error of p will be given by the square root of the reciprocal of the amount of information or $\sqrt{\frac{(1-p^2)(2+p^2)}{2n(1+2p^2)}}$.

A very common problem confronting the plant geneticist is one in which one of the two factor pairs being studied acts as a zygotic lethal when homozygous recessive, rendering the plant unclassifiable for the other factor pair in the lethal phenotypes. This results in the typical 9:3:4 ratio when the two factor pairs are inherited independently. Assuming that the *aB* and *ab* phenotypes die, or are indistinguishable, the expected frequency ratio in F_2 will be $1/4\ (2+p^2,\ 1-p^2,\ 1)$. The expectation in the $aB+ab$ class, being independent of p, will supply no information on linkage. Designating the observed numbers in the *AB* and *Ab* classes as a and b, as before, we may find the value of p which will satisfy the conditions of maximum likelihood. Multiplying the observed numbers in each class by the logarithm of the expected, and summing, we obtain, $a \log (2+p^2)+b \log (1-p^2)+(c+d) \log 1$. Maximizing this equation for p we obtain

$$\frac{2ap}{2+p^2}-\frac{2bp}{1-p^2}=0, \tag{III}$$

and the value of p can be calculated readily from $p=\sqrt{\frac{a-2b}{a+b}}$. This formula was first given by COLLINS (1924). The formula for the standard error of p can be calculated from the expression $\frac{2ap}{2+p^2}-\frac{2bp}{1-p^2}$. Differentiating again with respect to p, substituting the expected for the observed frequencies and changing signs of the terms we obtain the amount of information contained in the data in the form

$$\frac{3np^2}{(2+p^2)(1-p^2)}. \tag{IV}$$

The reciprocal of this, or $\frac{(2+p^2)(1-p^2)}{3np^2}$, will be the sampling vari-

ance of p and the square root of this quantity will be the standard error of p. By dividing the variance obtained when all classes are viable in F_2 by the above variance, we may find the efficiency of linkage determinations made when two classes are lethal compared with classifications in which four classes are viable. This is given by

$$\frac{(1-p^2)(2+p^2)}{2n(1+2p^2)} \div \frac{(1-p^2)(2+p^2)}{3np^2} = \frac{3p^2}{2(1+2p^2)}.$$

This tends toward zero as p tends toward zero, becomes .25 when p = .50 and rises to .50 when p = 1.00. It is obvious, therefore, that the error of linkage calculations based on repulsion crosses in which one character is lethal in the homozygous recessive condition is very high compared with determinations in which all classes are viable. Even in coupling crosses the relative accuracy does not exceed one-half that obtained when all classes are viable for any value of p. For close linkage in repulsion crosses the amount of information supplied by classification for only the two distinguishable phenotypes is extremely small for ordinary populations studied.

ROBERTSON, DEMING and KOONCE (1932) studied the linkage relations between the factor pairs *Ycyc* and *Fcfc* determining green vs. virescent seedlings and green vs. chlorina seedlings, respectively. Plants of the *ycyc* were lethal in the seedling stage rendering it impossible to classify the plants for the *Fcfc* factor pair when the *Ycyc* factor pair was homozygous recessive. The cross was one of repulsion. They observed in F_2

Phenotype	*Symbol*	*Number of plants*
Yc Fc	a	4165
Yc fc	b	1823
Yc Fc+*yc fc*	c+d	1853
Total	n	7841

The linkage intensity would then be given by

$$p = \sqrt{\frac{4165-2(1823)}{4165+1823}} = .2944 \text{ or } 29.44 \text{ percent crossing over.}$$

The amount of information contained in these data would be given by equation IV, or

$$\frac{3(7841)(.2944)^2}{\{1-(.2944)^2\}\{2+(.2944)^2\}} = 1069.74.$$

The standard error of p would be

$$\sqrt{\frac{1}{1069.74}} = .0306 \text{ or } 3.06 \text{ percent.}$$

A condition frequently encountered in F_2 classification is one in which the genetic constitution of the material is such that the AB and Ab phenotypes can not be separated, resulting in an expected 12:3:1 ratio when $p = .50$. For such cases the value of p satisfying the conditions of maximum likelihood will be given by

$$p = \sqrt{\frac{d}{c+d}}$$

with a standard error of p given by $\sqrt{\frac{1-p^2}{n}}$.

Compared with classifications in which all classes are distinguishable the above classification is 100 percent efficient when $p = 0$, falls to 75 percent efficiency when $p = .50$ and tends toward 50 percent as p tends toward 1.00.

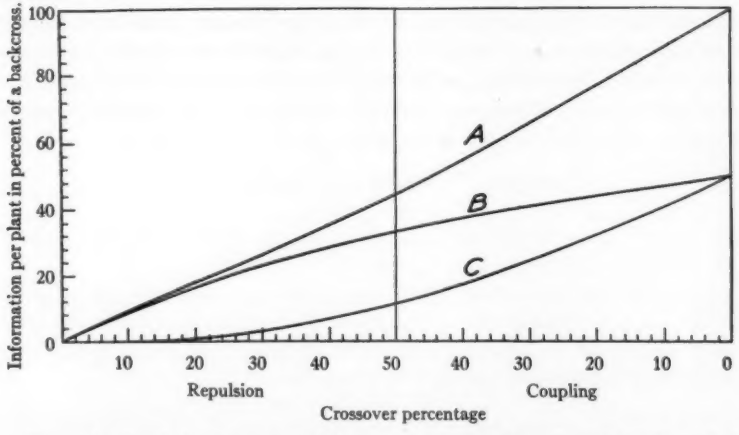

FIGURE 1.—Graph showing amount of information per plant, in percent of a backcross, in F_2 data when; (A) all classes are fully viable and distinguishable, (B) the AB and Ab phenotypes can not be separated, and (C) the aB and ab phenotypes can not be separated.

The relative accuracy of F_2 classifications when (1) all phenotypes are distinguishable, (2) the AB and Ab phenotypes are indistinguishable or (3) the aB and ab phenotypes are indistinguishable is shown graphically in figure 1. The information per plant grown and classified from these

three types of separations is given in percent of the information which would be supplied by a single plant from a backcross.

The accuracy of F_2 data in the coupling phase when all classes are viable, approaches the accuracy of a backcross as the recombination approaches zero. When the *aB* and *ab* phenotypes are indistinguishable for one of the factor pairs the amount of information supplied for close linkage by the F_2 repulsion phase is very small.

The estimation of the linkage from F_2 classification must be based on the ratio of the observed numbers of individuals in the different phenotypes. With the exception of the double recessive class the F_2 phenotypes are made up of two or more genotypes. By growing and classifying progenies of selfed F_2 plants in F_3 it is possible, with suitable numbers in the F_3 lines, to determine the genotype of each F_2 plant. The linkage may be estimated, then, from the frequency of occurrence of the various genotypes. The manner in which F_3 data may be used to supplement the F_2 will be discussed in some detail.

ESTIMATING LINKAGE INTENSITIES FROM F_3 DATA

In table 1 are given the expected frequencies, in terms of p, of the different genotypes which may be distinguished in progenies of selfed F_2 plants. Designating the probability of occurrence of the *AB*, *Ab*, *aB* and *ab* gametes as p, 1−p, 1−p and p, respectively, and assuming that crossing over occurs equally frequently in both male and female gametogenesis the probability of occurrence of the different genotypes is found quickly by multiplying the probability of the male by the female gametes.

TABLE 1

Frequency of occurrence of the nine genotypes expected from progenies of selfed F_2 plants.

	AA	*Aa*	*aa*
BB	p^2	$2p(1-p)$	$(1-p)^2$
Bb	$2p(1-p)$	$2p^2 \quad 2(1-p)^2$	$2p(1-p)$
bb	$(1-p)^2$	$2p(1-p)$	p^2

The genetic expectation in the *AaBb* genotype will depend on the genetic recombination which produced this genotype. If the recombination is one of coupling the expectation will be $2p^2$ and if it is repulsion it will be $2(1-p)^2$. The total expectation for the *AaBb* genotype will be $2p^2+2(1-p)^2$ or $2(1-2p+2p^2)$. In table 2 are given the symbols to be used to designate the observed frequency of the individuals of each genotype actually observed in an experiment.

We will consider first the separation of the "doubly dominant" F_2 progenies into genotypes by growing progenies of selfed F_2 plants in F_3.

"Doubly dominant" is used to denote the *AB* phenotypes; that is, plants in which at least one gene of each of the two factor pairs is dominant. We will assume also that the *AaBb* genotypes are not to be separated into two components depending on their origin from coupling or repulsion recombination. The expected frequency of the two kinds of *AaBb* genotypes combined will be, therefore, $2(1-2p+2p^2)$. The total expected frequency ratio in the 4 genotypes of the *AB* phenotype, it will be remembered, is $\frac{2+p^2}{4}$.

TABLE 2

Symbols used to designate the observed frequency of occurrence of individuals of various genotypes.

	AA	*Aa*	*aa*
BB	e	f	l
Bb	g	h i	m
bb	j	k	n

The value of p which will satisfy the conditions of maximum likelihood will be found by multiplying the observed frequencies in the different genotypes by the logarithm of the expected, summing and making this quantity a maximum, note being taken of the basis on which these are expected. Thus,

$$e \log \frac{p^2}{2+p^2} + (f+g) \log \frac{2p(1-p)}{2+p^2} + (h+i) \log \frac{2(1-2p+2p^2)}{2+p^2}$$

when maximized for p will give the equation of maximum likelihood in the form

$$\frac{2e+f+g}{p} - \frac{f+g}{1-p} - \frac{2(h+i)(1-2p)}{1-2p+2p^2} - \frac{(e+f+g+h+i)2p}{2+p^2} = 0. \qquad (V)$$

This formula, on substituting the actual numbers, found in an experiment, for e, f, g, h and i will yield the best possible value of p.

ROBERTSON et al (1930), in the linkage study referred to previously, grew and classified 746 F_3 progenies from as many F_2 plants of the *YcFc* phenotype. The genotype constitution of these plants was as follows:

Genotype	*Symbol*	*Number of F_3 lines*
Yc Yc Fc Fc	e	31
Yc yc Fc Fc	f	157
Yc Yc Fc fc	g	145
Yc yc Fc fc	h+i	413

Substituting the observed frequencies in equation V we obtain

$$\frac{364}{p}-\frac{302}{1-p}-\frac{826(1-2p)}{1-2p+2p^2}-\frac{1492p}{2+p^2}=0.$$

The exact value of p can be approached most rapidly by substituting likely values for p and interpolating. Thus, we may estimate the value of p near .30 and substitute in the formula given above. The result is illustrated below.

	p=.30	p=.29	p=.2994
$\frac{364}{p}$	1213.33	1255.17	1215.765
$-\frac{302}{1-p}$	−431.43	−425.35	−431.059
$-\frac{826(1-2p)}{1-2p+2p^2}$	−569.66	−589.80	−570.891
$-\frac{1492p}{2+p^2}$	−214.16	−207.61	−213.771
Total	−1.92	32.41	.044

The result of substituting p=.30 being −1.92 we assume that p is but slightly less than .30 and substitute p=.29; obtaining 32.41 as a total. The true value must give zero as a total so we interpolate by calculating .29+.01 (32.41÷34.33) and find p=.2994. Substituting p=.2994 we find the total deviation from zero to be .044. This serves as a check on the previous calculation as well as means of obtaining a more accurate value if one is required.

The amount of information contained in the data from this type of analysis in F_3 may now be calculated. To do so we differentiate equation V, that is,

$$\frac{2e+f+g}{p}-\frac{f+g}{1-p}-\frac{2(h+i)(1-2p)}{1-2p+2p^2}-\frac{(e+f+g+h+i)2p}{2+p^2}$$

with respect to p and obtain

$$-\left\{\frac{2e+f+g}{p^2}+\frac{f+g}{(1-p)^2}-\frac{2(h+i)4p(1-p)}{(1-2p+2p^2)^2}+\frac{2(e+f+g+h+i)(2-p^2)}{(2+p^2)^2}\right\}.$$

Substituting the expected for the observed frequencies we have

$$-\frac{2}{2+p^2}\left\{\frac{p(2-p)}{p^2}+\frac{2p(1-p)}{(1-p)^2}-\frac{2(1-2p+2p^2)4p(1-p)}{(1-2p+2p^2)^2}+\frac{(2-p^2)}{(2+p^2)}\right\}$$

which reduces to

$$-\frac{4(2-6p+3p^2+4p^3)}{p(1-p)(2+p^2)^2(1-2p+2p^2)}.$$

Changing signs we find the amount of information per F_2 plant, classified into genotypes in F_3, to be

$$\frac{4(2-6p+3p^2+4p^3)}{p(1-p)(2+p^2)^2(1-2p+2p^2)}. \qquad \text{(VI)}$$

The information supplied by n F_3 lines will be n times this quantity and the standard error of p will be the square root of the reciprocal of the amount of information or

$$\sqrt{\frac{p(1-p)(2+p^2)^2(1-2p+2p^2)}{4n(2-6p+3p^2+4p^3)}}.$$

Turning to the data of ROBERTSON, DEMING and KOONCE we find the numerical amount of information by substituting p = .2994 in equation VI, giving 3254.47. The standard error will be $\sqrt{\frac{1}{3254.47}}$ = .0175 or 1.75 percent.

Since the F_2 classification is concerned only with a separation of the phenotypes and the F_3 classification involves the separation of the doubly dominant (*AB*) phenotypes into their component genotypes the information contained in the latter will be supplementary to that contained in the former. In the data of ROBERTSON et al the best value of p for both F_2 and F_3 data will be found by combining the formulae given by the maximum likelihood solution for F_2 and F_3 classification; that is, equations III and V. Thus,

$$2p\left(\frac{4165}{2+p^2}-\frac{1823}{1-p^2}\right)+\frac{364}{p}-\frac{302}{1-p}-\frac{826(1-2p)}{1-2p+2p^2}-\frac{1492p}{2+p^2}=0.$$

Solving this by the method of approximation illustrated before we find the value of p which will satisfy best both the F_2 and F_3 data to be .2981 or 29.81 percent crossing over. The standard error to be attached to this linkage can be calculated readily from the total amount of information as

calculated from equations IV and VI. Since the F_2 supplied 1069.74 units of information and the F_3 supplied 3254.47, the total will be 4324.21. The standard error of p calculated for both F_2 and F_3 data will then be $\sqrt{\frac{1}{4324.21}} = .0152$ or 1.52 percent. The crossover percentage for both F_2 and F_3 data is, therefore, 29.81 percent with a standard error of 1.52 percent.

One other source of information remains in the F_3 data obtained from selfed plants of the doubly dominant F_2 phenotypes. This is the separation of the two types of "double heterozygotes," depending on their origin from coupling or repulsion recombination; where "double heterozygote" is used to denote plants heterozygous for both factor pairs. Since the expected frequency for coupling and repulsion, in the double heterozygous genotypes is $2p^2$ and $2(1-p)^2$, respectively, the value of p which will satisfy best the conditions of maximum likelihood will be given by

$$h \log \frac{2p^2}{2(1-2p+2p^2)} + i \log \frac{2(1-p)^2}{2(1-2p+2p^2)}$$

which will be a maximum when

$$\frac{2h}{p} - \frac{2i}{1-p} + \frac{2(h+i)(1-2p)}{1-2p+2p^2} = 0. \qquad \text{(VII)}$$

The amount of information, per F_3 line classified, in this separation is found to be given by

$$\frac{4}{(1-2p+2p^2)^2}. \qquad \text{(VIII)}$$

This amount of information is supplementary to both the F_2 and that given in equation VI for the simple separation of the doubly dominant F_2 phenotypes into homozygous and single and double heterozygotes and can be added to them if this separation is made.

The number of plants per F_3 line needed in order to separate the two types of double heterozygotes will depend on the linkage intensity. The linkage may be calculated for each doubly heterozygous progeny and the separation into coupling or repulsion recombination made on this basis. Due to the error of random sampling the calculated values of p will vary around the true value as origin. A point may be calculated, however, from which the deviation of the true p and q (where $q = 1-p$) will be twice their standard error. Letting such a midpoint be p' it will be possible to classify each F_3 doubly heterozygous progeny as arising through coupling or repulsion recombination depending on whether the calculated value of p

is greater or less than this midpoint, p′. When all classes are viable the number of individuals needed, per F_3 family, in order that the difference between the true value of p or q and this midpoint shall be twice the standard error of p or q will be given by

$$\sqrt{n} = 2\left\{\sqrt{\frac{(1-p^2)(2+p^2)}{2(1+2p^2)}} + \sqrt{\frac{(1+q^2)(2+q^2)}{2(1+2q^2)}}\right\} \Big/ (q-p).$$

From this we find that 25 plants per F_3 line are sufficient for this separation when the crossover percentage is less than 22 percent. As the crossover percentage increases to 25 percent 33 plants would be required, for 30 percent crossing over 54 plants per F_3 line are needed, at 35 percent 97 are required, at 40 percent 223 are needed and at 45 percent the number rises to 898. It will be only for linkages which do not greatly exceed 22 percent that this separation is worthwhile.

The midpoint or p′ from which the separation is to be made of the coupling or recombination double heterozygotes, with all classes viable, will be given by

$$p' = p + \frac{2}{\sqrt{n}}\sqrt{\frac{(1-p^2)(2+p^2)}{2(1+2p^2)}}.$$

The value of p′ for varying percentages of crossing over is shown graphically in figure 2.

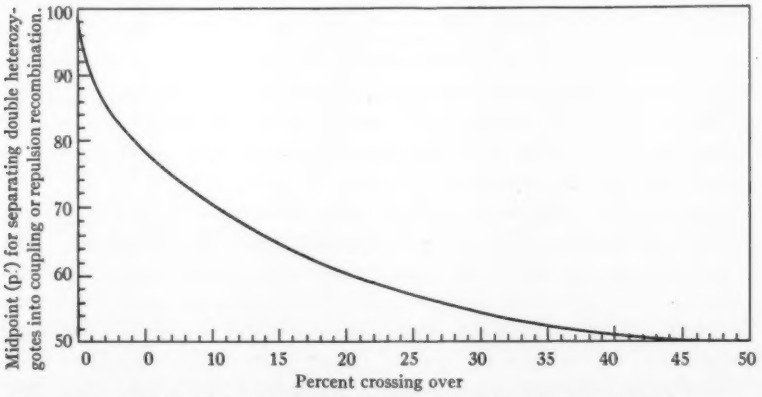

FIGURE 2.—Graph showing value of midpoint (p′) to be used in separating double heterozygotes, by F_3 classification, as arising through coupling or repulsion recombination.

This midpoint, p′, is .9008 for 1 percent crossing over, falls to .7845 for

5 percent crossing over, to .7046 for 10 percent crossing over and to .6057, .5453, .5112 and .5000 as the crossover percentage increases to 20, 30, 40 and 50 percent respectively. Due to the fact that the error of the linkage intensities increases as p decreases, p' will exceed .5 for all values of the crossover percentage less than 50 percent.

We may consider next the classification of the "singly dominant" selfed progenies of F_2 plants into homozygotes and heterozygotes as a result of F_3 classification. "Singly dominant" is used to denote F_2 plants homozygous recessive for one factor pair and having at least one gene of the other factor pair in the dominant condition. We will consider only the *Ab* phenotype since the expectation for the *aB* phenotype is the same. The equation of maximum likelihood would take the form

$$j \log \frac{(1-p)^2}{1-p^2} + k \log \frac{2p(1-p)}{1-p^2}$$

which, when maximized for p would give

$$\frac{k}{p} - \frac{2j+k}{1-p} + \frac{(j+k)2p}{1-p^2} = 0 \qquad \text{(IX)}$$

as the formula for determining the value of p from such a separation. Differentiating again with respect to p, substituting the expected for the observed frequencies and changing signs we have the amount of information per F_2 plant classified by F_3 separation in the form

$$\frac{2}{p(1+p)(1-p^2)} . \qquad \text{(X)}$$

The standard error of p, based on n such F_3 lines would be

$$\sqrt{\frac{p(1+p)(1-p^2)}{2n}} .$$

The value of p which will satisfy the conditions of maximum likelihood when both F_2 and F_3 data are used can be obtained readily by simply combining in an additive manner the terms on the left hand side of the equality sign applicable to the particular separations involved and setting the entire quantity equal to zero. This was illustrated in the simple case of the data of ROBERTSON et al. The standard error to be attached to the value of p found from a combination of data from different classifications is obtained readily from the total amount of information; the latter being the sum of the information obtained from the different sources individually. In table 3 is illustrated the additive manner in which such information combines.

TABLE 3

Illustration of the manner of combining information from different types of classification when all phenotypes are viable and can be separated.

TYPE OF SEPARATION	INFORMATION PER PLANT	NUMBER OF PLANTS	TOTAL INFORMATION
F_2	$\frac{2(1+2p^2)}{(1-p^2)(2+p^2)}$	n	$\frac{2n(1+2p^2)}{(1-p^2)(2+p^2)}$
F_3 separation of *AB* phenotypes ignoring origin of double heterozygotes	$\frac{4(2-6p+3p^2+4p^3)}{p(1-p)(2+p^2)^2(1-2p+2p^2)}$	$\frac{n(2+p^2)}{4}$	$\frac{n(2-6p+3p^2+4p^3)}{p(1-p)(2+p^2)(1-2p+2p^2)}$
F_3 separation of double heterozygotes	$\frac{4}{(1-2p+2p^2)^2}$	$\frac{2n(1-2p+2p^2)}{4}$	$\frac{2n}{1-2p+2p^2}$
F_3 separation of *Ab* phenotypes	$\frac{2}{p(1+p)(1-p^2)}$	$\frac{n(1-p^2)}{4}$	$\frac{n}{2p(1+p)}$
F_3 separation of *aB* phenotypes	$\frac{2}{p(1+p)(1-p^2)}$	$\frac{n(1-p^2)}{4}$	$\frac{n}{2p(1+p)}$
Total	$\frac{2}{p(1-p)}$	n	$\frac{2n}{p(1-p)}$

Multiplying the information per plant by the number of plants expected for each type of separation we obtain the total amount of information; as given in the last column in the table. Adding the different amounts of information obtainable from F_2 classification and the separation of the doubly and singly dominant F_2 progenies into genotypes by F_3 classification we obtain $\frac{2n}{p(1-p)}$ as the total amount of information or $\frac{2}{p(1-p)}$ as the amount of information per F_2 plant completely classified. This is twice the amount of information given by a backcross, as expected, since the number of crossovers in both male and female gametogenesis are known. In actual experiments the numerical amount of information, obtained from the various sources is added and the standard error to be attached to the crossover percentage calculated from a combination of several types of separation determined from the total amount of information.

COMPARISON OF F_2 AND F_3 DATA WHEN ALL GENOTYPES ARE FULLY VIABLE

From doubly dominant F_3 progenies the information is of three types, (1) from small families of about 25 plants it will always be possible to separate the homozygotes from the single and double heterozygotes. The ratio of the amount of information from this source to that from a single F_2 plant is

$$\frac{4(2-6p+3p^2+4p^3)}{p(1-p)(2+p^2)^2(1-2p+2p^2)} \div \frac{2(1+2p^2)}{(1-p^2)(2+p^2)} = \frac{2(2-6p+3p^2+4p^3)(1+p)}{p(1+2p^2)(2+p^2)(1-2p+2p^2)}.$$

When p is less than .08 this value exceeds 25, which shows the advantage of growing F_3 progenies from double dominants when p is less than .08 for this first purpose. In other words, F_3 classification of the above type will give more information per plant grown than will the F_2 when there is less than 8 percent crossing over as determined from repulsion crosses.

The second source of information (2) expected from doubly dominant F_3 progenies when the families are sufficiently large or the linkage sufficiently close for the two types of double heterozygotes to be separated, since the proportion of the two genotypes depends on the linkage, will add to the precision of our estimate by an amount $\frac{4}{(1-2p+2p^2)^2}$ for each double heterozygote discriminated or

$$\frac{4}{(1-2p+2p^2)^2} \times \frac{2(1-2p+2p^2)}{2+p^2} = \frac{8}{(2+p^2)(1-2p+2p^2)}$$

for each double dominant examined.

Once the two types of double heterozygotes have been separated the third source of information (3) obtained will be ordinary F_2 data obtained from the segregation of the double heterozygotes in F_3.

Adding the information available from the three sources from complete classification of the doubly dominant F_3 progenies such classification will give more information per plant grown than will the F_2 when p is .26 or less. For higher values of p F_2 classification will give a greater amount of information per plant grown and classified than would F_3 classification.

Twenty-five plants would be sufficient to separate the singly dominant (*Ab* or *aB* phenotypes) into genotypes by F_3 classification. The ratio of information from singly dominant F_3 progenies to that given by F_2 will be

$$\frac{2}{p(1+p)(1-p^2)} \div \frac{2(1+2p^2)}{(1-p^2)(2+p^2)} = \frac{2+p^2}{p(1+p)(1+2p^2)}.$$

This exceeds 25 when p is less than .075 so that for closer repulsion than this, F_3 progenies of this kind would supply more information per plant grown than would an F_2 of equal size, considering 25 plants per F_3 line sufficient.

Since 25 plants per line would be sufficient to separate the double dominants into homozygotes and single or double heterozygotes and to separate the single dominants into homozygotes or heterozygotes it would be of interest to determine which would give the greater amount of information.

Dividing $\frac{4(2-6p+3p^2+4p^3)}{p(1-p)(2+p^2)^2(1-2p+2p^2)}$ by $\frac{2}{p(1+p)(1-p^2)}$ we find that this is less than unity when p lies between .18 and .69. Therefore it would be more profitable to grow and classify, in F_3, singly dominant progenies than to classify doubly dominant progenies into homozygotes and single and double heterozygotes when p is greater than .18 and less than .69, that is, when the crossover percentage is greater than 18 percent in repulsion or 31 percent in coupling. For closer linkage equation VI would supply more information than would IX.

In figure 3 is given a comparison of the relative accuracy of linkage calculations made from F_3 data when (A) the separation of the doubly dominant progenies is made completely, including the separation of the double heterozygotes as to origin in coupling or repulsion, (B) the genotypic constitution of the single heterozygotes is determined and (C) separation of the double dominants into homozygotes and single and double heterozygotes only, ignoring the separation of the double heterozygotes as to origin in coupling or repulsion.

The lines for B and C in the above graph cross at the points $p = .18$ and $p = .69$. The value of separating the two types of double heterozygotes is shown by the difference between curves A and C.

COMPARISON OF F_2 AND F_3 CLASSIFICATION WHEN THE HOMOZYGOTES FOR ONE OF THE CHARACTERS DIE

When two of the four F_2 phenotypes are inviable when homozygous recessive for one of the factor pairs and do not permit of classification for the other character in these phenotypes, complete classification would be possible only in the remaining two classes, giving a theoretical frequency ratio of $1/4\{2+p^2, 1-p^2, 1\}$. The two classes which die would supply no information on linkage. Since the amount of information available from F_2 separation is very small for close linkage in repulsion it would be expected that F_3 classification would here be extremely valuable.

With two classes of F_2 phenotypes indistinguishable and only small progenies grown in F_3, failure to recover the *aB* phenotypes in F_3 from selfed double heterozygotes due to chance would render it impossible to distinguish the *AaBb* F_2 genotype from the *AABb* by F_3 classification. If the requirement of five expected plants in the *aB* phenotype (resulting from selfing the F_2 *AaBb* genotype) be set up as a minimum to guard

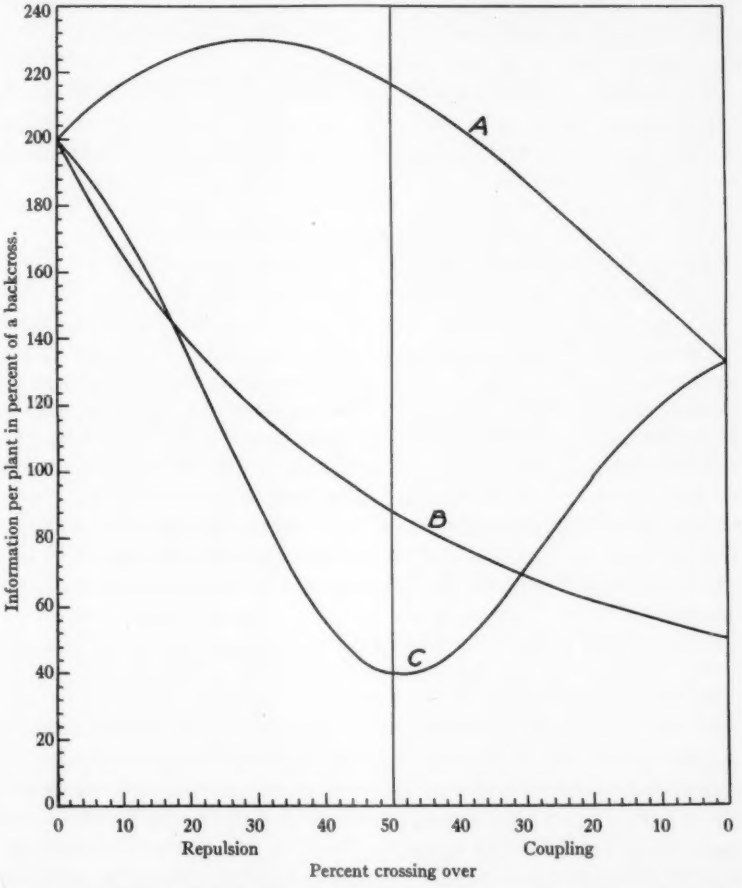

FIGURE 3.—Graph showing amount of information, in percent of a backcross, per F_2 plant whose genotype is determined by growing selfed progenies in F_3 when; (A) the double dominants are completely classified, (B) the single dominants are separated into homozygotes and heterozygotes and (C) the double dominants are separated into homozygotes and single and double heterozygotes only, ignoring the separation of the double heterozygotes into two components depending on origin.

against chance loss of all plants of this phenotype, this number would be expected from 25 individuals per F_3 line only when p is less than the number given by $\frac{25(1-p^2)}{4}=5$ or when p is less than .447. For higher values of p a larger F_3 progeny would need to be grown in order to separate the double heterozygote from the *AABb* genotype, the number required from any given value of p being given by substituting x for 25 in the above equation and solving for x.

The number of plants per F_3 line necessary to separate the double heterozygotes into the two genotypes as to origin in coupling or repulsion in order that the deviation of p and q (=1+p) from the midpoint should be twice the standard error of p or q, respectively, would be given by

$$\sqrt{n}=2\left\{\sqrt{\frac{(1-p^2)(2+p^2)}{3p^2}}+\sqrt{\frac{(1-q^2)(2+q^2)}{3q^2}}\right\}\Big/(q-p).$$

The number of plants required (n) would be 250 per F_3 line for 20 percent crossing over and rises as the crossover percentage increases or decreases from this point. Apparently, then, it would be useless to contemplate separation of the double heterozygotes into the two types. When the doubly dominant F_3 progenies are tested, therefore, the separation could be made only for the homozygous dominants and single and double heterozygotes, without attempting to separate the latter as to origin. This could be accomplished with 25 plants per F_3 line when p is less than .447, while a greater number of plants would be required when p exceeds this point.

Such being the case the doubly dominant progenies would be more profitable when p is less than .18 and the single dominants would be more profitable when p exceeds .18. With all classes viable the double dominants again became more profitable than the single dominants when p exceeded .69, that is, less than 31 percent crossing over in coupling. With two of the four classes of phenotypes dying, or indistinguishable, the number of plants per F_3 line for the separation of the double dominants when p exceeds .69 would exceed 25 by an amount which would render the single dominants the most profitable on the basis of plants grown, for all values of p in excess of .18.

Comparing the amount of information in the separation of the double dominants into homozygotes and single and double heterozygotes by F_3 classification with that available from F_2 separation we have

$$\frac{4(2-6p+3p^2+4p^3)}{p(1-p)(2+p^2)^2(1-2p+2p^2)} \div \frac{3p^2}{(1-p^2)(2+p^2)} = \frac{4(2-6p+3p^2+4p^3)(1+p)}{3p^3(2+p^2)(1-2p+2p^2)}.$$

This exceeds 25 when p is less than 31.5 percent so that for closer linkage than this F_3 classification of this type would be more profitable than an F_2 population of the same number of plants, assuming 25 plants grown per F_3 line.

Comparing the singly dominant F_3 progenies with the F_2 we obtain

$$\frac{2}{p(1+p)(1-p^2)} \div \frac{3p^2}{(1-p^2)(2+p^2)} = \frac{2(2+p^2)}{3p^3(1+p)}.$$

This exceeds 25 when p is less than .347 which shows the F_3 classification of this type is more profitable, per plant grown, than the F_2 for smaller values of p. It is apparent that when two phenotypes are indistinguishable in F_2 the error is so much greater than when all phenotypes may be discriminated that F_3 classification is more profitable over a wider range of p.

SUMMARY

1. Formulae are presented for estimating the linkage intensity from F_2 data when two of the four classes are indistinguishable. The relative accuracy of such estimations of linkage compared with estimations made when all four classes are distinguishable is given.

2. The manner in which data from F_3 progenies, of selfed F_2 plants, may be used in calculating linkages is illustrated.

3. Doubly dominant F_3 progenies will supply more information per plant grown when separated into homozygotes and single and double heterozygotes alone, than will F_2 classification when p is less than 8 percent in repulsion. When the double heterozygotes are separated as well and the additional F_2 information from the F_3 lines is utilized such double dominant progenies, classified into genotypes in F_3, supply more information per plant grown than the F_2 when p is less than 26 percent in repulsion.

4. Singly dominant F_3 progenies are more valuable than an F_2 of equal members when p is less than 7.5 percent in repulsion.

5. When all genotypes are viable the doubly dominant F_3 progenies separated into homozygotes and single and double heterozygotes supply more information than single dominant F_3 progenies when p is less than .18 or greater than .69.

6. When the homozygous recessives, for one of the characters, die or are indistinguishable F_3 separation of the double dominants into homo-

zygotes and single and double heterozygotes proves much more profitable than growing an F_2 of equal size when p is less than .315. Such doubly dominant F_3 progenies are more profitable than single dominant progenies when p is less than .18. For looser linkage in repulsion, or in coupling crosses, the single dominants would be preferable.

7. The manner of estimating the linkage intensity from F_2 data and F_3 data of various kinds is illustrated. The standard error to be attached to this crossover percentage is calculated from the total amount of information.

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RELATION OF THE DIFFERENTIAL FERTILIZATION GENES, *Ga ga*, TO CERTAIN OTHER GENES OF THE *Su-Tu* LINKAGE GROUP OF MAIZE[1]

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The *Ga*, or "gamete" gene of maize can be studied only or principally by its disturbance of normal Mendelian ratios of contrasted characters differentiated by genes linked with it. The amount of this disturbance can be used as a measure of the intensity of linkage between *Ga* and other genes of the *su-Tu* group.

Disturbance of the 3:1 ratio of starchy, *Su*, to sugary, *su*, endosperm has been most studied. In one of the early papers on Mendelian inheritance, CORRENS (1902) reported that, although crosses between most starchy and sugary varieties gave an F_2 ratio of 3:1 for starchy and sugary kernels, a popcorn with pointed kernels, when crossed with sugary races, gave only 16 percent of sugary kernels in F_2. That *Su* and *su* segregated normally was shown by reciprocal crosses of F_1 with the sugary parent, which gave approximately 50 percent sugary kernels. From this CORRENS concluded that the deficiency of sugary kernels in F_2 is due to selective fertilization.

JONES (1924) reported that crosses of rice popcorn with sugary maize gave 16.2 percent sugary kernels in F_2, while other starchy races crossed with sugary gave approximately 25 percent sugary kernels. Backcrosses to sugary gave about 50 percent sugary, as CORRENS had reported. From counts of starchy and sugary kernels on the butt and tip ends of ears, JONES concluded that the mechanism of this selective fertilization was differential pollen-tube growth. The writer (EMERSON 1925) suggested that this assumed differential pollen-tube growth is due not to the *Su su* genes themselves but to some gene linked with *Su*. MANGELSDORF and JONES (1926) called this accessory gene the "gamete" factor, *Ga*. Since *Ga* and *ga* pollen exhibit differential fertilization when the pistils have *Ga* either homozygous or heterozygous the gene is regarded as dominant. Backcrosses of F_1 on the sugary parent carrying the homozygous recessive allelomorph, *ga*, show little or no selective fertilization.

By dividing the silks of *Ga* carrying ears and pollinating those of one side with *Ga* and those of the other side with *ga* pollen, DEMEREC (1929) obtained results which indicate that differential fertilization is due to cross-sterility of *ga* pollen on *Ga* plants. The writer's repeated attempts to de-

[1] Paper No. 200, Department of Plant Breeding, CORNELL UNIVERSITY, Ithaca, New York.

termine whether differential fertilization involving *Ga* and *ga* is due to differential pollen-tube growth or to cross-sterility have given somewhat contradictory and, therefore, confusing results. Fortunately an understanding of the mechanism of differential fertilization is not essential in a consideration of the relation of *Ga ga* to other genes of the *su-Tu* linkage group.

CROSSES BETWEEN VARIOUS TYPES OF STARCHY AND OF SUGARY MAIZE

In the course of the writer's studies of the relation of *Ga* and *ga* to the *Su-su* pair, records of crosses of many different types of starchy maize with

TABLE 1

Starchy and sugary kernels obtained from self-pollinated plants of the crosses indicated.

ITEM NUMBER	GENERATION	PERCENT SU OF PRECEDING GENERATION	NUMBER OF EARS	NUMBER OF KERNELS			PERCENT SUGARY
				STARCHY	SUGARY	TOTAL	
Common starchy crossed with common sugary. F_1 genotype: *ga Su/ga su*							
1	F_2		834	203,700	68,089	271,789	25.1
2	F_3	F_2 normal	148	31,051	10,173	41,224	24.7
Rice pop crossed with common sugary. F_1 genotype: *Ga Su/ga su*							
3	F_2		363	111,368	19,798	131,166	15.1
4			96	26,340	5,009	31,349	16.0
5	F_3	F_2 low	81	20,171	6,505	26,676	24.4
6			16	2,858	1,608	4,466	36.0
7			52	10,646	2,147	12,793	16.8
8		F_3 low	63	14,286	4,681	18,967	24.7
9			6	977	522	1,499	34.8
10	F_4	F_3 normal	75	16,526	5,600	22,126	25.3
11			4	272	55	327	16.8
12		F_3 high	39	7,501	2,465	9,966	24.7
13			32	6,124	3,669	9,793	37.5
Common starchy crossed with sugary derivatives of rice pop. F_1 genotype: *ga Su/Ga su*							
14	F_2		263	60,704	33,775	94,479	35.7
15			21	4,994	876	5,870	14.9
16	F_3	F_2 high	99	23,067	7,932	30,999	25.6
17			101	19,953	10,820	30,773	35.2
Rice pop crossed with sugary derivatives of rice pop. F_1 genotype: *Ga Su/Ga su*							
18	F_2		197	60,670	20,200	80,870	25.0
19	F_3	F_2 normal	9	1,601	550	2,151	25.6

several varieties of sugary maize have accumulated. The starchy types used in these crosses included different varieties of dent, flint, flour, and pop maize and of crosses between them. The sugary types used included several varieties of sweet corn, of crosses between them, and of sugary derivatives from various starchy-sugary crosses. Records of progenies obtained from self-pollination of individuals of various generations of the several crosses are summarized in table 1.

All of these starchy types, except rice pop and derivatives of it, when crossed with any of the sugary lots except derivatives of rice pop, have given normal ratios of starchy to sugary, approximately 25 percent sugary (table 1, items 1, 2). The genotype of heterozygous starchy plants of these crosses is assumed to be *ga Su/ga su*.

In strong contrast to these results are the records of crosses of rice pop and its starchy derivatives with many of the same lots of sugary maize employed in the crosses noted above. Heterozygous starchy plants of these rice pop crosses, *Ga Su/ga su*, have consistently given low percentages of sugary, with perhaps somewhat greater variation than that observed in crosses involving *ga ga*. The average for the F_2 generation was 15.1 percent sugary (table 1, item 3). Some of the starchy derivatives of rice pop were evidently *Ga ga*, for about half of their F_2 progenies had approximately 25 percent sugary while the other half showed a wide departure from 25 percent.

Not only was the F_2 percentage of sugary in these crosses involving *Ga ga* different from that of the crosses involving *ga ga*, but the F_3 behavior also was different. F_3 progenies from *Su-su* F_2's of the latter crosses were all of one class, the percentage of sugary varying about 25 as a mean. The F_3 progenies of *Su-su* F_2's of the *Ga-ga* crosses, on the contrary, fell into three classes with low, normal, and high sugary percentages. The usual variation resulted doubtless in some overlapping of classes, but in general they were readily separable, varying about their respective means of 16.0, 24.4, and 36.0 percent sugary (table 1, items 4, 5, 6).

Heterozygous starchy kernels of the low sugary F_3 class repeated in F_4 the behavior of F_2 starchy kernels in F_3. There were again three fairly distinct classes of progeny with mean percentages of sugary of 16.8, 24.7, and 34.8 (table 1, items 7, 8, 9). Numerically these three classes, with F_3 and F_4 combined, were as 148:144:22 for low, normal, and high sugary, respectively. The high sugary class presumably arises from crossing over, producing the genotype *Ga su/ga Su*, and hence of relatively infrequent occurrence.

The F_4 progenies from *Su-su* plants of the normal sugary F_3 class showed normal sugary only, the mean percentage being 25.3 (table 1, item 10).

The high sugary F_3 lot gave the same three F_4 classes of low, normal, and

high sugary as did the low sugary F_3's. The mean percentages of sugary also were similar, being 16.8, 24.7, and 37.5, respectively (table 1, items 11, 12, 13). But the numerical relation of the low and high classes was reversed, low, normal, and high having the relation of 4:39:32, respectively. Here low sugary is a crossover class, *Ga Su/ga su*.

The normal sugary class in each F_3 and F_4 group must also have come from crossovers resulting in *Ga Su/Ga su*, or *ga Su/ga su*, or both. The latter, however, as will appear from evidence yet to be presented, should be relatively rare. It should, therefore, be readily possible to obtain sugary stocks of the type *Ga su*.

Crosses of all types of starchy maize, *ga Su*, except rice pop and its derivatives, with sugary derivatives of rice pop, *Ga su*, have given consistently high sugary percentages in F_2, with a mean percentage of 35.7 (table 1, item 14). When these sugary derivatives are *Ga ga*, there occur in F_2, of course, both normal and high sugary progenies in nearly equal numbers.

Heterozygous starchy kernels from high sugary ears of F_2 of this cross repeat in F_3 the behavior in F_4 of similar kernels of high sugary F_3 ears of the preceding cross (compare items 11, 12, 13 with 15, 16, 17 of table 1). Here again low, normal, and high sugary classes were observed, the mean percentages of sugary being 14.9, 25.6, and 35.2. The relative number of progenies of the three classes also was of the same general order. These F_4 and F_3 lots together were as 25:138:133 for low, normal, and high sugary, respectively.

Crosses of rice pop or its starchy derivatives, *Ga Su*, with sugary derivatives of rice pop, *Ga su*, have given uniformly normal sugary percentages in F_2 and F_3. Of course, when one or the other parent, or both, had *Ga ga*, the resulting progenies were normal and low, normal and high, or all three together. The percentage of sugary in F_2 was 25.0 and in F_3, with smaller numbers, 25.6 (table 1, items 18, 19).

Both CORRENS (1902) and JONES (1924) reported that crosses of rice pop with various sugary races, showing when selfed a low percentage of sugary kernels, exhibited normal segregation of starchy and sugary, with approximately 50 percent of sugary kernels, when backcrossed reciprocally with the sugary parents. Obviously the sugary parents of these crosses were *ga*, and the results indicated that differential fertilization does not occur when the silks to which *Ga-ga* pollen is applied do not have *Ga*. MANGELSDORF and JONES (1926) backcrossed plants of the genotype *Ga Su/ga su* with the rice pop parent, *Ga Su*, and noted in the following generation the numbers of *Su-Su* and *Su-su* plants. Almost equal numbers of the two classes were observed, 213 *Su Su* and 207 *Su su*, when rice pop furnished the pollen for the backcrosses. When, however, rice pop was used as the female parent of the backcrosses, the ratio of *Su Su* to *Su su*

was 353 to 88. In short, *su* pollen of F_1 plants had functioned in the production of only 20 percent of the kernels.

A more direct test of differential fertilization in backcrosses is obtained by backcrossing to *Ga-su* as well as to *ga-su* plants. Accordingly, a number of plants, which when selfed gave low, normal, or high percentages of sugary, were crossed—some of them reciprocally—with both *ga su* and *Ga su*. The results are presented in table 2.

TABLE 2

Backcrosses of various genotypes involving Ga ga and Su su by and on ga su and Ga su.

CONSTITUTION OF HETEROZYGOTES AND TYPE OF POLLINATION	NUMBER OF HETEROZYGOUS PLANTS	NUMBER OF KERNELS			PERCENT SUGARY
		STARCHY	SUGARY	TOTAL	
ga Su/ga su					
Self-pollinated	50	14,252	4,736	18,988	24.9
Crossed by *ga su* ♂	6	853	807	1,660	48.6
Crossed by *Ga su* ♂	14	1,917	1,798	3,715	48.4
Crossed on *ga su* ♀	41	3,771	3,992	7,763	51.4
Crossed on *Ga su* ♀	50	5,579	5,643	11,222	50.3
Ga Su/Ga su					
Self-pollinated	10	4,272	1,412	5,684	24.8
Crossed by *ga su* ♂	4	581	593	1,174	50.5
Crossed by *Ga su* ♂	3	479	471	950	49.6
Crossed on *ga su* ♀	2	191	214	405	52.8
Crossed on *Ga su* ♀	5	547	573	1,120	51.2
Ga Su/ga su					
Self-pollinated	53	19,498	3,296	22,794	14.5
Crossed by *ga su* ♂	13	2,518	2,451	4,969	49.3
Crossed by *Ga su* ♂	16	3,663	3,535	7,198	49.1
Crossed on *ga su* ♀	45	5,328	5,796	11,124	52.1
Crossed on *Ga su* ♀	44	8,860	3,844	12,704	30.3
Ga su/ga Su					
Self-pollinated	24	5,805	3,494	9,299	37.6
Crossed by *ga su* ♂	4	541	509	1,050	48.5
Crossed by *Ga su* ♂	11	1,425	1,412	2,837	49.8
Crossed on *ga su* ♀	14	2,010	1,903	3,913	48.6
Crossed on *Ga su* ♀	17	1,768	3,729	5,497	67.8

Only two of these backcrosses exhibited large departures from 50 percent sugary kernels. Plants of the genotypes *Ga Su/ga su* and *Ga su/ga Su* when used as pollen parents in crosses with *Ga su* gave respectively 30.3 and 67.8 percent sugary. These are departures in opposite directions from 50 of 19.7 and 17.8, respectively, corresponding to the deviations from 25 percent of 10.5 and 12.6 in the progenies derived from self-pollination.

RELATION OF LINKAGE OF *Ga ga* WITH OTHER GENES AND OF THE PERCENT OF FUNCTIONING *Ga* AND *ga* POLLEN TO DISTORTED RATIOS

It has been assumed that the differential fertilization genes *Ga ga* disturb the normal *Su-su* ratios because of linkage of these genes. Obviously the magnitude of the disturbance of ratios of dominant to recessive genes is related to the intensity of linkage between them and the *Ga ga* pair. If there were no such linkage the disturbing effect of *Ga ga* would be zero and their existence unsuspected. That they do not distort ratios of genes in linkage groups other than the *su-Tu* group was shown by adding to the genotypes *Ga Su/ga su* and *Ga su/ga Su* certain well known aleurone, endosperm, and plant-color genes in a heterozygous condition.

That the distortion of ratios of dominant to recessive is related also to the percentage of *Ga* and *ga* pollen that functions in fertilization is equally obvious. If *Ga* and *ga* pollen each fertilized 50 percent of the kernels, no disturbance of the ratios of other dominant and recessive genes would result no matter how close the linkage between them and *Ga ga*. This is what occurs when *Ga ga* pollen is used on *ga* plants. *Ga* and *ga* are not, in such cases, differential fertilization genes.

It has been pointed out by MANGELSDORF and JONES (1926) that any given deviation from a normal Mendelian ratio might be due to any one of many different combinations of linkage intensity and percentage of functioning *Ga* and *ga* pollen. If, therefore, we knew the percentages of *Ga* and *ga* pollen effective in fertilization, we could readily determine the percentage of recombination necessary to produce an observed deviation from the normal Mendelian ratio.

MANGELSDORF and JONES (1926) made such an evaluation of the relative effectiveness of *Ga* and *ga* pollen on *Ga* silks. Two curves were constructed, one from the observed percentage of sugary kernels, 16.2, and the other from the observed percentage of defective, de_1, kernels, 17.2, on self-pollinated plants heterozygous for *Ga ga* and for *Su su* or $De_1\ de_1$. The curve for percentage of sugary was so constructed that, at any point on it, the ordinate, read as recombination percentage between *Ga* and *su*, and the abscissa, read as percentage of functioning *ga* pollen, should give the observed percentage of sugary. The curve for percentage of defective kernels was constructed in the same way. The problem was then to determine at what point on the axis of abscissas an ordinate could be erected to intersect the two curves at points whose recombination values, converted into map distance and combined, would equal the map distance corresponding to the observed recombination percentage between *su* and de_1. The recombination percentage for *su* and de_1 was taken as 39[2] corre-

sponding to a map distance of approximately 50. Since the ordinate found best to fit this value intersected the axis of abscissas at approximately 19.6 percent of functioning *ga* pollen, the percentages of recombination were indicated to be 21.2 for *Ga* and *su* and 24.3 for *Ga* and de_1.

From the published data of MANGELSDORF and JONES (1926) with the addition of more recent unpublished data by the same authors involving a total of 14,677 kernels, the recombination percentage of de_1 and *su* is calculated as approximately 47. This corresponds to a map distance of 75 units according to HALDANE's (1919) table; and the present indication is that the actual map distance between de_1 and *su* is somewhat more than 75 units. The percentages of defective in a total of 4518 kernels on high defective ears and in a total of 1022 kernels on low defective ears (MANGELSDORF and JONES 1926) were respectively 32.0 and 16.8. The weighted mean departure from 25 percent is 7.2, corresponding to an average of 17.8 percent defectives. The data presented in table 1 show 15.4 percent sugary in a total of 181,505 kernels on ears of the low sugary class and 35.7 percent in 141,010 kernels on ears of the high sugary class. The deviations from 25 percent are, therefore, 9.6 and 10.7, respectively. The weighted average deviation is 10.2, corresponding to a percent of sugary of 14.8.

On the basis of these data, namely, 47 percent of recombination between *su* and de_1, 17.8 percent de_1, and 14.8 percent sugary, the method of MANGELSDORF and JONES (1926), with the aid of their table 9, gives as the percentage of functioning *ga* pollen on *Ga* silks 2.5, and as the recombination percentages between *Ga* and de_1 34.9, and between *Ga* and *su* 28.5. The corresponding map distances are 42.4 and 32.6 totaling 75 which is taken as the map distance for 47 percent recombination between de_1 and *su*. Since the actual map distance is probably greater, rather than less, than 75, it is interesting to note that this method of calculation gives, for zero percent of functioning *ga* pollen, recombination percentages of 35.6 for *Ga* and de_1 and 29.6 for *Ga* and *su*, the combined map distances of which are about 78.0. In so far, therefore, as this method is at all reliable, it may be concluded that the percentage of functioning *ga* pollen in competition with *Ga* pollen on *Ga* silks is from zero to 2.5.

A more direct method for an evaluation of the relative effectiveness of *Ga* and *ga* pollen on *Ga* silks is the pollination of *Ga* plants with a known mixture of *Ga* and *ga* pollen. In such a mechanical mixture of pollen, one variable is eliminated, namely, the crossovers between *Ga ga* and genes linked with them, which occur in the hybrid mixture of pollen of a heterozygous plant.

[2] The frequency distribution reported by MANGELSDORF and JONES (1926) of 601 De_1 *Su*, 238 De_1 *su*, 247 de_1 *Su*, 64 de_1 *su* gives a recombination percentage of 44.0 as computed by aid of IMMER's (1930) tables.

Such pollen-mixture tests were reported by MANGELSDORF and JONES (1926) but not used for the purpose of determining the relative effectiveness of *Ga* and *ga* pollen in hybrid mixtures. A mixture of pollen of rice pop, *Ga Su*, with pollen of a variety of sugary maize, *ga su*, was applied to the silks of both of the types that furnished the pollen. The starchy type had white and the sugary type yellow endosperms, so that the kernels resulting from cross-pollination could be distinguished from those resulting from self-pollination. From the pollination of the sugary plants 6435 kernels were obtained of which 4233, or 65.8 percent, were sugary. This indicates that there was an abundance of viable *ga-su* pollen in the mixture. The same pollen mixture used on rice pop gave a total of 12,690 kernels of which only 75, or 0.59 percent, were yellow. In short, *ga* pollen functioned in competition with *Ga* pollen on *Ga* silks in the production of only a little more than one-half of one percent of the kernels.

Similar tests by DEMEREC (1929) gave almost identical results. Pollen from *ga* plants with purple aleurone and shrunken endosperm was mixed with pollen from *Ga*, (popcorn) plants with colorless aleurone and non-shrunken endosperm, each type thus having one dominant and one recessive character by which kernels produced by cross-pollination could be distinguished from those produced by self-pollination. Pollination of *ga* plants with this mixture resulted in 1189 kernels of which 654, or 55.0 percent, were fertilized with *ga* pollen. Pollination of *Ga* plants with the same mixture gave 1690 kernels of which only 13, or 0.77 percent, were fertilized with *ga* pollen. Silks of *Ga* plants pollinated with pollen from several different varieties of *ga* plants, without competition from *Ga* pollen, resulted in the production of only 39 kernels on ears estimated to have had 7090 ovules, a percentage of only 0.55.

The writer's tests with mechanical mixtures of *Ga* and *ga* pollen were conducted because of the bearing they were expected to have on the problem of the relative effectiveness of *Ga* and *ga* pollen in hybrid mixtures. Endosperm color and composition were used to identify kernels resulting from the different kinds of pollen. In all but one test mixtures of only two kinds of pollen were used, one carrying *Ga* and the other *ga*, while in the one test four kinds of pollen were mixed, involving all the four combinations of *Ga* and *ga* with *Su* and *su*. In half of the tests the pollen mixtures were applied to *ga su* and *Ga su* silks only, while in the other half they were put on *ga Su* and *Ga Su* as well. In each test approximately equal quantities of the different kinds of pollen were mixed thoroughly, then divided, and each part applied at once to one or other of the kinds of plants to be pollinated. Since it is impossible to be certain that any mixture contains equal numbers of viable pollen grains, of the stocks used, all mixtures were used on *ga* plants, on which no differential fertilization is expected,

TABLE 3

Summary of tests of pollen mixtures applied to maize silks of different genotypes.

NUMBER OF TESTS SUMMARIZED	POLLEN MIXTURE	GENOTYPE OF SILKS	GENOTYPE OF KERNELS PRODUCED				
			NUMBER			PERCENT	
			Ga	*ga*	TOTAL	*su*	*ga*
			Su	*su*			
3	*Ga Su*+*ga su*	*ga su*	1,089	1,627	2,716	59.9	59.9
		Ga su	1,958	85	2,043	4.2	4.2
			su	*Su*			
4	*Ga su*+*ga Su*	*ga su*	968	1,044	2,012	48.1	51.9
		Ga su	3,261	94	3,355	97.2	2.8
			su	*su*			
1	*Ga su*+*ga su*	*ga su*	208	270	478	..	56.5
		Ga su	727	41	768	..	5.3
			Su	*Su*			
2	*Ga Su*+*ga Su*	*ga su*	344	132	476	..	27.7
		Ga su	1,707	10	1,717	..	0.6
		ga Su	477	320	797	..	40.2
		Ga Su	1,447	24	1,471	..	1.6
			su	*Su*			
2	*Ga su*+*ga Su*	*ga su*	185	67	252	73.4	26.6
		Ga su	1,185	18	1,203	98.5	1.5
			Su	*Su*			
		ga Su	576	177	753	..	23.5
		Ga Su	670	25	695	..	3.6
			Su	*su*			
3	*Ga Su*+*ga su*	*ga su*	371	653	1,024	63.8	63.8
		Ga su	1,909	99	2,008	4.9	4.9
			Su	*Su*			
		ga Su	476	644	1,120	..	57.5
		Ga Su	797	71	868	..	8.2
			Su+*su*	*Su*+*su*			
1	*Ga Su*+*ga su* + *Ga su*+*ga Su*	*ga su*	605	1,029	1,634	..	63.0
		Ga su	2,478	186	2,664	..	7.0
			Su	*Su*			
		ga Su	348	523	871	..	60.0
		Ga Su	201	21	222	..	9.5
			Ga	*ga*			
16	*Ga*+*ga*	*ga*	5,647	6,486	12,133	..	53.5
		Ga	16,340	674	17,014	..	3.96

in order to determine quantitatively the actual composition of the mixtures used in the tests with *Ga* plants. The results of these tests are summarized in table 3.

It will be noted that the percentages of kernels fertilized by *ga* pollen on *ga* plants varied from 23.5 to 63.8 with an average, for the 12,133 ker-

nels, of a little more than 50. In strong contrast to these results, *ga* pollen functioned on *Ga* plants in the production of from 0.6 to 9.5 percent of the kernels, with an average, for the 17,014 kernels, of only about 4.0 percent. The 4 percent of functioning *ga* pollen on *Ga* plants shown by these tests is greater than indicated by similar tests of MANGELSDORF and JONES and of DEMEREC which showed only about 0.6 and 0.8 respectively. It is also somewhat in excess of the 2.5 percent calculated from deviations from 3:1 ratios of De_1 to de_1 and *Su* to *su* by the method of MANGELSDORF and JONES (1926). The conclusion to be drawn from all these tests is that on the average *ga* pollen on *Ga* plants functions in the production of from almost zero to about 4 percent of the kernels.

Inability to determine the percentage of functioning *ga* pollen more closely affects very little the results of calculations of linkage intensity between *Ga ga* and *Su su*. Thus, the recombination percentage with zero percent of functioning *ga* pollen is 29.6, with 2.5 percent 28.5 and with 4.0 percent 27.8. Duplicate linkage tests involving other genes often give results as diverse as these.

With no functioning *ga* pollen, the percentage of sugary or of starchy kernels in a backcross on *Ga su* plants should equal the percentage of recombination. Table 2 shows 30.3 percent sugary from *Ga su*×*Ga Su/ga su* and 67.8 percent sugary, or 32.2 percent starchy from *Ga su*×*Ga su/ga Su*. The weighted average of these records is 30.9 percent for a total of 18,201 kernels. This is only 1.3 greater than the percentage of recombination calculated, on the basis of no functioning *ga* pollen, from the data given in table 1 involving 322,515 kernels.

Since, with the methods so far discussed, it is necessary to evaluate the relative effectiveness of *Ga* and *ga* pollen before recombination percentages can be determined, a method that is independent of *Ga ga* may be of interest. If r:s:s:r be taken as the gametic series for any percentage of crossing over and p and q as the respective percentages of effective *Ga* and *ga* pollen, the several genotypes resulting from self-pollination of *Ga Su/ga su* or *Ga su/ga Su* should occur with the frequencies shown below:

Su Su—

$$\left.\begin{array}{l} Ga\ Su/Ga\ Su = p\ r^2 \\ Ga\ Su/ga\ Su = (p+q)rs \\ ga\ Su/ga\ Su = q\ s^2 \end{array}\right\} = p\ r^2+(p+q)rs+q\ s^2$$

su su—

$$\left.\begin{array}{l} Ga\ su/Ga\ su = p\ s^2 \\ Ga\ su/ga\ su = (p+q)rs \\ ga\ su/ga\ su = q\ r^2 \end{array}\right\} = q\ r^2+(p+q)rs+p\ s^2$$

Total *Su Su* and *su su* $= (p+q)\ r^2+2(p+q)\ rs+(p+q)\ s^2$

Su su

$$\left.\begin{array}{l} Ga\ Su/Ga\ su = 2p\ rs \\ ga\ Su/ga\ su = 2q\ rs \\ Ga\ Su/ga\ su = (p+q)r^2 \\ Ga\ su/ga\ Su = (p+q)\ s^2 \end{array}\right\} = (p+q)\ r^2 + 2(p+q)\ rs + (p+q)\ s^2$$

From this theoretical frequency distribution, it may be noted that the *Su-su* genotypes which should produce low, normal, and high sugary percentages have the following frequencies, respectively: $(p+q)r^2 : 2(p+q)rs : (p+q)s^2$, or, when simplified, $r^2 : 2rs : s^2$. In the coupling phase of linkage between *Ga ga* and *Su su*, giving rise to a low sugary percentage, $r > s$; in the repulsion phase, resulting in high sugary, $r < s$. With a given percentage of crossing over, the values of r and s are reversed in coupling and repulsion. Therefore, the value of the first and the third terms of the frequency distribution, $r^2 + 2rs + s^2$, are reversed while the value of the second term remains constant for coupling and repulsion phases of linkage.

The relative values of the three terms depend not alone on the linkage phase, but also on the intensity of linkage. As crossing over approaches 50 percent, $r = 1$ and $s = 1$, these values approach 1:2:1; as crossing over percentage approaches zero, $r = 1$ and $s = 0$ or $r = 0$ and $s = 1$, the values approach 1:0:0 or 0:0:1. The relative numbers of progenies showing low, normal, and high sugary percentages produced from *Su su* kernels of low sugary and of high sugary ears should, therefore, give an indication of the percentage of crossing over between *Ga ga* and *Su su* without involving the problem of the ratio of functioning *Ga* to *ga* pollen.

Table 1 shows that, from *Su su* kernels of low sugary and from high sugary ears, there were produced a total of 610 progenies exhibiting low, normal, and high sugary percentages in the following numbers:

	Progenies			
Parent ears	Low sugary	Normal sugary	High sugary	Total
Low sugary	148	144	22	314
High sugary	25	138	133	296

As indicated above, the first and third terms are reversed in coupling and repulsion phases of linkage. When rearranged so that the first term consists of the individuals that are like their respective parent ears, the observed distribution is as given below. For comparison, distributions calculated on the basis of various percentages of crossing over are included.

		Deviation from 25 percent sugary				
		Like parent ear	None	Reverse of parent ear	Total	Value of χ^2
Observed		281	282	47	610	
Calculated for recombination						
percentage of—	28	316	246	48	610	9.2
	29	307	252	51	610	6.1
	30	299	256	55	610	4.9
	31	290	261	59	610	4.4
	32	282	266	62	610	4.5
	33	271	271	68	610	7.3

The first and third terms, r^2 and s^2, of the observed distribution, give 16.76 and 6.86 as values of r and s, respectively, or a crossover percentage of 29.0. It will be recalled that, from the percentage of sugary seeds given in table 1 and by the method of MANGELSDORF and JONES (1926), the percentage of functioning *ga* pollen was calculated as 2.5 and the percent of recombination between *Ga* and *su* as 28.5. The frequency distribution, calculated from 29 percent recombination, does not give a good fit with the observed distribution, $\chi^2 = 6.1$ and $P = 0.048$. A considerably better fit is shown by the distribution calculated for 31 percent recombination, $\chi^2 = 4.4$, $P = 0.114$. This is approximately the recombination percentage, 30.9, given by the backcross data of table 2, when calculated on the basis of no functioning *ga* pollen.

The method here under consideration is independent of the relative effectiveness of *Ga* and *ga* pollen. Its principal disadvantage is the impracticability of dealing with large numbers. None of the frequency distributions calculated by this method fits very well the observed distribution. But do calculated frequency distributions based on the percentage of sugary kernels fit observed frequencies well? When the percentages of starchy and sugary kernels alone are used, conclusions are based on two classes where three exist, namely, *Su Su*, *Su su*, and *su su*.

Since, as shown by the theoretical frequency distribution given above, the heterozygous, *Su su*, kernels equal the sum of the homozygous kernels, *Su Su* and *su su*, any values of r, s, and p, q which result in a deviation upward or downward from 25 percent *su su* should bring about an inverse deviation downward or upward from 25 percent *Su Su*. The low sugary lots of table 1 averaged 15.4 percent sugary, the normal sugary lots 25.0 percent sugary, and the high sugary lots 35.7 percent sugary. From

starchy kernels of low, normal, and high sugary ears, therefore, the three classes should have the following relation:

Parent ears	Progenies			Total
	Su Su	*Su su*	*su su*	
Low sugary	34.6	50.0	15.4	100.0
Normal sugary	25.0	50.0	25.0	100.0
High sugary	14.3	50.0	35.7	100.0

The percentages of homozygous starchy among all starchy kernels of the low, normal, and high sugary lots, therefore, should be, respectively, 40.9, 33.3, and 22.2. A comparison of calculated frequencies, based on these percentages, with observed frequencies is as follows:

Parent ears	Progenies		Total	Percent *Su Su*
	Su Su	*Su su*		
Low sugary—				
Observed	227	352	579	39.2
Calculated	237	342	579	40.9
Difference	−10	+10	0	−1.7
Normal sugary—				
Observed	98	210	308	31.8
Calculated	103	205	308	33.3
Difference	−5	+5	0	−1.5
High sugary—				
Observed	94	353	447	21.0
Calculated	99	348	447	22.2
Difference	−5	+5	0	−1.2

This comparison shows a remarkably close fit of observed frequencies with frequencies calculated from the percentages of sugary kernels. It would seem, therefore, that methods of calculating linkage intensity between *Ga ga* and *Su su*, based on observed percentages of sugary kernels, should give reliable results.

RELATION OF *Ga ga* TO GENES OTHER THAN *Su su*

As noted earlier in this paper, records published by MANGELSDORF and JONES (1926) show that in a high defective lot of maize with a total of

4518 kernels 32.0 percent were defective, *de*$_1$, and in a low defective lot with 1022 kernels 16.8 percent were *de*$_1$. Rice pop, or a derivative of it, was involved in both of these lots. The weighted mean deviation from 25 percent of the two lots is 7.2, corresponding to an average percentage of 17.8 *de*$_1$ kernels. On the assumption that no *ga* pollen functions on *Ga* silks, this percentage should result from 35.6 percent of recombination between *Ga ga* and *De*$_1$ *de*$_1$. The percentage of recombination calculated on the basis of 4 percent of effective *ga* pollen is 34.3. It may be concluded, then, that the recombination value is not far from 35 percent.

Another defective, *de*$_{16}$, when crossed with a derivative of rice pop, showed a marked deviation from the usual 25 percent defective (WENTZ 1925). In a total of 1784 kernels 17.3 percent were defective. This corresponds to recombination percentages of 34.6 and 33.2 with zero and with 4 percent of effective *ga* pollen, respectively. The recombination value for *Ga ga* and *De*$_{16}$ *de*$_{16}$, therefore, may be given as approximately 34 percent.

As indicated earlier in this paper, the records of MANGELSDORF and JONES, including 14,677 kernels, indicate a recombination percentage of about 47 for *de*$_1$ and *su*. WENTZ (1925) reported a recombination percentage of 3.2 for *de*$_{16}$ and *su* calculated from counts of 28,276 kernels. Since the writer's records indicate approximately 29 percent of recombination between *Ga* and *su*, the order of these genes is probably: *de*$_1$, *Ga*, *su*, *de*$_{16}$.

Tassel seed-5 plants with sun-red plant color, *ga Ts*$_5$ *B*, were crossed with rice pop which was dilute sun-red and had normal tassels, *Ga ts*$_5$ *b*. Pollen of the F_1 plants was applied to silks of plants having the genotypes *ga ts*$_5$ *b* and *Ga ts*$_5$ *b*, with the following results:

	Backcross Progeny				
F_1 Backcrossed on	*Ts*$_5$	*ts*$_5$	Total	Percent *Ts*$_5$	Percent *b*
ga ts$_5$ *b*	401	449	850	47.2	45.3
Ga ts$_5$ *b*	244	570	814	30.0	47.4

Although there was a deficiency of *Ts*$_5$ in the progeny of the backcross on *ga ts*$_5$ *b*, it is less than that of the recessive, *b*. When the F_1 was backcrossed on *Ga ts*$_5$ *b*, however, the deficiency of *Ts*$_5$ was much more while that of *b* was less than for the other backcross. The *B-b* pair is known to belong to another linkage group and the percentage of *b*-plants, therefore, should not be disturbed by *Ga*. The gene *Ts*$_5$ is known to belong to the *su-Tu* group. With no functioning *ga* pollen, the percent of *Ts*$_5$ should be the percent of recombination between *Ga* and *Ts*$_5$.

TABLE 4

Backcrosses of ga ts_5 su/ga Ts_5 Su and Ga ts_5 su/ga Ts_5 Su on ga ts_5 su and Ga ts_5 su.

GENOTYPE OF F_1 AND OF ♀ PARENT OF BACKCROSS	NUMBER OF INDIVIDUALS					RECOMBINATIONS		PERCENTAGE		
	Su Ts_5	*Su* ts_5	*su* Ts_5	*su* ts_5	TOTAL	NUMBER	PERCENT	*su*	Ts_5	*b*
F_1 $\frac{ga\ ts_5\ su}{ga\ Ts_5\ Su}$										
1—*ga* ts_5 *su*	158	22	28	155	363	50	13.6	50.4	51.2	..
2—*Ga* ts_5 *su*	161	28	21	154	364	49	13.5	48.1	50.0	..
F_1 $\frac{Ga\ ts_5\ su\ b}{ga\ Ts_5\ Su\ B}$										
3—*ga* ts_5 *su b*	213	38	42	232	525	80	15.2	52.2	48.6	48.0
4—*Ga* ts_5 *su b*	167	107	28	624	926	135	14.6	70.4	21.1	47.4
Total	699	195	119	1165	2178	314	14.4	..	..	..

Common starchy tassel seed-5 plants, *ga* Ts_5 *Su*, were crossed both with common sugary, *ga* ts_5 *su*, and with sugary derivatives of rice pop, *Ga* ts_5 *su*. The F_1's of both lots were then backcrossed on *ga* ts_5 *su* and on *Ga* ts_5 *su*. As for the crosses noted above, some of the F_1's were *B b*. The results are presented in table 4. The data reported in this table exhibit no wide departure from the expected 50 percent of *su*, Ts_5 or *b*, except for the backcross *Ga* ts_5 *su*×*Ga* ts_5 *su*/*ga* Ts_5 *Su* (table 4, backcross 4). For that cross 70.4 percent of the plants were produced from *su* kernels, and 21.1 percent of the plants were Ts_5. These wide deviations from 50 percent, however, did not disturb the percentage of recombination between *su* and Ts_5 which is 14.6 for this culture and 14.4 for the average of all four cultures. The percentages of *su*, 70.4, and of Ts_5, 21.1, indicate, with no functioning *ga* pollen, percentages of recombination of 29.6 for *Ga su* and 21.1 for *Ga* Ts_5; or, with 4 percent functioning *ga* pollen, 27.8 and 18.5, respectively. The recombination percentages may be taken, therefore, as 14 for *su* Ts_5, 20 for *Ga* Ts_5, and 29 for *Ga su*, and the order of the genes as *Ga* Ts_5 *su*.

If the percentages of recombination between *Ga su* and between *Ga* Ts_5 were calculated from the percentages of *su* and of Ts_5 observed in backcross 3 of table 4, 52.2 and 48.6, their respective values would be found to be 47.8 and 48.6. But the pollen for backcross 3 was taken from the same F_1 plants as that for backcross 4 from which the values 29.6 and 21.1 are calculated. Since the pollen for backcross 3 was applied to *ga* silks, *ga* as well as *Ga* pollen should have functioned in the production of 50 percent of the kernels. As the percentage of functioning *ga* pollen approaches 50,

the deviations from normal percentages of *su* or Ts_5 approach zero; and, with zero deviation, 50 percent of recombination is indicated for any percentage of functioning *ga* gametes from zero to 50. These calculations, therefore, reveal just what was to have been expected.

EYSTER (1921) reported 29 percent of recombination between sugary and tunicate, *Tu*. The writer has backcross data from 4206 individuals, involving both coupling and repulsion phases of linkage, which also indicate 29 as the percentage of recombination. A cross of common sugary tunicate, *ga su Tu*, with rice pop, *Ga Su tu*, backcrossed on *Ga su tu* plants, gave the following distribution:

Genotype	Backcross progeny				
of F_1	*Su Tu*	*Su tu*	*su Tu*	*su tu*	*Total*
Ga Su tu/ga su Tu	281	552	188	92	1113

These data show 33.5 percent of recombination between *su* and *Tu*, and 25.2 percent *su* and 42.1 percent *Tu*. These percentages, with no effective *ga* pollen, become the percentages of recombination between *Ga* and *su* and between *Ga* and *Tu*, respectively. With 4 percent functioning *ga* pollen, the respective percentages are 23.0 and 41.4. The order of these genes, therefore, is *Ga su Tu*.

The several combinations of the six genes considered here have been shown by two-point tests to have approximately the following percentages of crossing over:

de_1 *Ga* 35, de_1 *su* 47, de_{16} *Ga* 34, de_{16} *su* 3, *Ga su* 29, *Ga* Ts_5 20, *Ga Tu* 42, *su* Ts_5 14, *su Tu* 29. The order of the genes and their approximate spacing, therefore, is apparently as follows:

de_1 35 *Ga* 20 Ts_5 14 *su* 3 de_{16} 26 *Tu*.

THREE-POINT TESTS INVOLVING *Ga ga*

Backcross data involving two other genes linked with *Ga* can be arranged in three-point tables from which can be determined the percentages of crossing over between any two of the three genes and the coincidence of crossing over. It is, however, essential to know, at least approximately, the percentages of functioning *Ga* and *ga* pollen. It has been shown, both by the method of MANGELSDORF and JONES (1926) and by the method of mixed pollen, that *ga* pollen in competition with *Ga* pollen on *Ga* silks functions in the fertilization of from 0 to 4 percent of the kernels. Both these percentages, therefore, will be used in the examples to be given here.

For any order of the three genes, let

N = the total number of individuals

w = the number of non-crossovers

x = the number of single crossovers in region 1

y = the number of single crossovers in region 2
z = the number of double crossovers
p = the percentage of functioning *Ga* pollen
q = the percentage of functioning *ga* pollen.

The data presented earlier for *Ga su Tu* will be used in illustrating the method under consideration. The observed frequency distribution is:

Su Tu	*Su tu*	*su Tu*	*su tu*	Total
281	552	188	92	1113

These data will be considered first on the assumption that 4 percent of *ga* pollen functions. It is known from the parents crossed to produce the F_1 generation whether *Ga* is linked with the dominant or recessive member of the *Su su* and *Tu tu* pairs, but, for purposes of illustration, this information will be disregarded. Since the observed frequencies show that *Su* and *tu* individuals are in excess of *su* and *Tu* ones, respectively, *Ga* must be linked with *Su* and *tu*, and *ga* with *su* and *Tu*. This eliminates all but three of the twelve possible F_1 genotypes. The observed frequencies show also that the ratio of *Su*:*su* deviates much more from the normal 1:1 ratio than does the ratio of *Tu*:*tu*. This indicates that *Ga ga* must be closer to *Su su* than to *Tu tu*; and this fact eliminates one of the three F_1 genotypes, leaving as possibilities only these two:

$$\frac{Ga\ Su\ tu}{ga\ su\ Tu} \quad \text{and} \quad \frac{Su\ Ga\ tu}{su\ ga\ Tu}.$$

These will be considered in the order given. Then:

$$\begin{aligned} Su\ Tu &= py + qz = 281 \\ su\ tu &= qy + pz = 92 \\ \hline y + z &= 373 \\ y &= 373 - z \\ z &= 373 - y. \end{aligned}$$

Substituting for y in the *Su Tu* equation,

$$\begin{aligned} &p(373 - z) + qz = 281 \\ &373p - pz + qz = 281 \\ &(p - q)z = 373p - 281 \\ &z = (373p - 281)/p - q \\ &\quad = 77.08/0.92 = 83.78 \\ &z/N = 83.78/1113 = 7.5 \text{ percent.} \end{aligned}$$

Substituting for z in the *Su Tu* equation and solving,

$$py+q\,(373-y)=281$$
$$y=289.22,\ y/N=26.0 \text{ percent}$$

Similarly,

$$Su\ tu=pw+qx=552$$
$$su\ Tu=qw+px=188$$
$$w+\ x=740$$
$$w=740-x$$
$$x=740-w.$$

Substituting in the *Su tu* equation for w and for x and solving,

$$p\,(740-x)+qx=552$$
$$pw+q\,(740-w)=552$$
$$x=172.17,\ x/N=15.5 \text{ percent}$$
$$w=567.83.$$

The recombination percentages are:

$$Ga\ Su\quad 15.5+\ 7.5=23.0$$
$$Su\ Tu\quad 26.0+\ 7.5=33.5$$
$$Ga\ Tu\quad 15.5+26.0=41.5.$$

The genotype chosen for illustration, *Ga Su tu/ga su Tu*, is in agreement with the calculated recombination percentages. When the other of the two supposedly possible genotypes, *Su Ga tu/su ga Tu*, is chosen, identically the same recombination percentages are obtained by the method of calculation used here, but such an arrangement of the genes is impossible with the recombination percentages found.

On the assumption that no *ga* pollen functions, the calculation of crossover percentages is greatly simplified. On that assumption, p = 1 and q = 0, and the equations become:

$$Su\ Tu=py+qz=y=281.\ y/N=25.2 \text{ percent}$$
$$su\ tu=qy+pz=z=92.\ z/N=8.3 \text{ percent}$$
$$Su\ tu=pw+qx=w=552.$$
$$su\ Tu=qw+px=x=182.\ x/N=16.9 \text{ percent}.$$

Three-point tables can be constructed from these data from *Ga su tu* × *Ga Su tu/ga su Tu*, as follows:

PERCENTAGE OF FUNCTIONING *Ga* AND *ga* POLLEN, p AND q	NON-CROSSOVERS		SINGLE CROSSOVERS, REGION 1		SINGLE CROSSOVERS, REGION 2		DOUBLE CROSSOVERS		TOTAL
	Ga Su tu *ga su Tu*		*Ga su Tu* *ga Su tu*		*Ga Su Tu* *ga su tu*		*Ga su tu* *ga Su Tu*		
	pw	qw	px	qx	py	qy	pz	qz	N
p=0.96, q=0.04	545	23	165	7	278	12	80	3	1113
	568		172		290		83		
			15.47 percent		25.99 percent		7.53 percent		

Recombination percentages: *Ga Su* 23.00, *Su Tu* 33.52, *Ga Tu* 41.46
Coincidence=0.9767

	Ga Su tu	*Ga su Tu*	*Ga Su Tu*	*Ga su tu*	
	w	x	y	z	
p=1, q=0	552	188	281	92	1113
		16.89 percent	25.25 percent	8.26 percent	

Recombination percentages: *Ga Su* 25.15, *Su Tu* 33.51, *Ga Tu* 42.14
Coincidence=0.9801

In the same way, the data from *Ga* ts_5 *su*×*Ga* ts_5 *su*/*ga* Ts_5 *Su* (table 4, backcross 4) are arranged in the following three-point table:

PERCENTAGE OF FUNCTIONING *Ga* AND *ga* POLLEN, p AND q	NON-CROSSOVERS		SINGLE CROSSOVERS, REGION 1		SINGLE CROSSOVERS, REGION 2		DOUBLE CROSSOVERS		TOTAL
	Ga ts_5 *su* *ga* Ts_5 *Su*		*Ga* Ts_5 *Su* *ga* ts_5 *su*		*Ga* ts_5 *Su* *ga* Ts_5 *su*		*Ga* Ts_5 *su* *ga* ts_5 *Su*		
	pw	qw	px	qx	py	qy	pz	qz	N
p=0.96, q=0.04	618	26	141	6	106	4	24	1	926
	644		147		110		25		
			15.87 percent		11.88 percent		2.70 percent		

Recombination percentages: *Ga* Ts_5 18.57, Ts_5 *su* 14.58, *Ga su* 27.75
Coincidence=0.9972

	Ga ts_5 *su*	*Ga* Ts_5 *Su*	*Ga* ts_5 *Su*	*Ga* Ts_5 *su*	
	w	x	y	z	
p=1, q=0	624	167	107	28	926
		18.03 percent	11.56 percent	3.02 percent	

Recombination percentages: *Ga* Ts_5 21.05, Ts_5 *su* 14.58, *Ga su* 29.59
Coincidence=0.9840

That coincidence of crossing over is practically unity, even for the relatively short map distances involved in the data for *Ga* Ts_5 *su*, suggests

the possibility that *Ga* and *su* may be on opposite sides of the spindle fiber insertion region.

It is of interest to compare the percentages of recombination for these *su-Tu* and Ts_5-*su* data as calculated by this three-point method with those determined directly from a chart based on deviations from normal ratios and constructed by the method of Mangelsdorf and Jones (1926). The comparison is given below both for zero and for 4 percent of functioning *ga* pollen.

	Functioning *ga* pollen			
	Zero percent		4 percent	
	Deviation method	3-point method	Deviation method	3-point method
su-Tu data				
Ga su	25.2	25.2	23.0	23.0
Ga Tu	42.1	42.1	41.4	41.5
Ts_5-*su* data				
Ga Ts_5	21.1	21.1	18.5	18.6
Ga su	29.6	29.6	27.8	27.8

SUMMARY

The effect of the genes *Ga ga* in causing deviations from normal 3:1 and 1:1 ratios is discussed, the results of other investigators showing the relation of *Ga ga* to De_1 de_1, De_{16} de_{16}, *Su su* are reviewed, and data are presented on the relations of *Ga ga* to *Su su*, Ts_5 ts_5, and *Tu tu*. Methods of evaluating the effectiveness of *Ga ga* and of determining recombination percentages are discussed. The relations of the six genes considered are summarized in terms of their linear order and approximate spacing on the genetic map, as follows: de_1 35 *Ga* 20 Ts_5 14 *su* 3 de_{16} 26 *Tu*.

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INTERLOCKING OF BIVALENT CHROMOSOMES IN TRADESCANTIA

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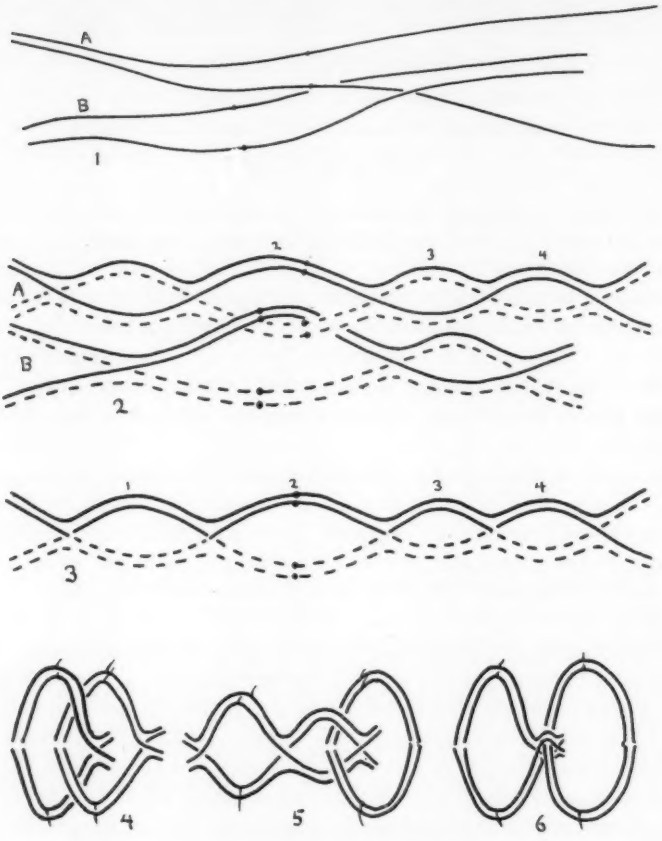
Interlocking of bivalent chromosomes at meiosis has been described in several genera of plants and animals. In favorable cases it may be seen at pachytene (Dendrocoelum, GELEI 1921) and is clearly shown at diakinesis and metaphase in Stenobothrus (BELAR 1928) and Salamandra (SCHREINER, see BELAR 1928). Interlocking occurs frequently in certain species of Oenothera (CLELAND 1922, CATCHESIDE 1931, et al.), Campanula (GAIRDNER and DARLINGTON 1931), and Tradescantia (SAX and ANDERSON 1933). In most genera interlocking of non-homologous bivalents is rare, if it occurs at all.

The types of chromosome interlocking and the relative frequency of these types provide evidence of value in interpreting the mechanism of chiasma formation and the subsequent behavior of chiasmata at meiosis. Because of the theoretical problems involved, a thorough analysis has been made of the more critical types of chromosome interlocking in Tradescantia.

Before describing chromosome interlocking in Tradescantia and other genera, the various types of interlocking will be described in relation to current theories of chiasma formation and behavior. At the time of pairing of homologous chromosomes, one homologue may be included between the two homologues of another bivalent, as shown in diagram 1. Such interlocking might occur if the chromosomes begin pairing at the ends, as indicated in the diagram, or if they pair first at the spindle fiber attachment points, which are indicated by dots in the figure. At pachytene the homologous chromosomes would be paired throughout their length with the exception of a short region where interlocking has occurred. Presumably such interlocking between non-homologous chromosomes could occur at any locus.

As the diplotene nodes and internodes are formed, three types of interlocking may be obtained. The two bivalents may be interlocked at the proximal or spindle fiber internodes, or the proximal loop of one bivalent may be locked with a distal loop of the other bivalent, or two distal loops may be interlocked. Proximal interlocking is shown in diagrams 2 and 4, while proximal-distal interlocking is shown in diagrams 5 and 6. The interlocking of two distal loops has not been observed in Tradescantia. Inter-



DESCRIPTION OF DIAGRAMS

DIAGRAM 1.—Interlocking of two non-homologous bivalents, A and B, at the time of pairing. The dots represent the spindle fiber attachment points.

DIAGRAM 2.—Interlocking at pachytene, showing the relation of the chromatids at the internodes on the classical theory of chiasma formation. Proximal interlocking, involving the internodes containing the fiber attachment points is shown. Distal interlocking could occur only at internode 4 of bivalent A and could not occur in bivalent B.

DIAGRAM 3.—Chiasma formation at diplotene on the partial chiasmatypy hypothesis. Interlocking could occur at any internode, and if at random, interlocking of distal internodes should be as frequent as proximal interlocking.

DIAGRAM 4.—Proximal interlocking at metaphase after reduction and terminalization of chiasmata.

DIAGRAM 5.—Distal interlocking.

DIAGRAM 6.—Distal interlocking of the type described in Oenothera and Campanula, but not found in Tradescantia.

locking involving a distal internode of one bivalent with the proximal loop of another will be referred to as distal interlocking.

As CATCHESIDE (1931) has pointed out, the types of interlocking will depend on the method of chiasma formation. If chiasmata are caused by the alternate opening-out of sister and non-sister chromatids, the "classical theory," interlocking can occur only at the reductional internodes (2 and 4, diagram 2). Only proximal interlocking is possible unless three chiasmata are formed on the same side of the spindle fiber attachment point. Distal interlocking could occur in only one of the four internodes of chromosome A and could not occur at all in chromosome B. GAIRDNER and DARLINGTON (1931) state that interlocking should occur between chromatids if the classical theory of chiasma formation is correct, but this suggestion is so obviously untenable that it need not be considered here (see SAX and ANDERSON 1933).

If chiasmata are the result of previous crossovers and only sister chromatids are associated at early diplotene, then interlocking could occur at any internode. According to the partial chiasmatypy theory, interlocking could occur at any one of the four internodes shown in diagram 3, and if interlocking is at random, distal interlocking should be at least as frequent as proximal interlocking.

The types of interlocking at diakinesis and metaphase will depend not only on the mechanism of chiasma formation, but also on the behavior of the chiasmata between early diplotene and these later stages. In the plant genera known to possess interlocked chromosomes, the chiasma frequency is from three to five per bivalent at diplotene and is reduced to about two at metaphase. On the classical theory of chiasma formation, chiasmata may be eliminated by cancellation or by breaking (SAX 1932), and in some cases chiasmata may pass off the ends of the chromosomes before metaphase. According to DARLINGTON'S interpretation of the partial chiasmatypy theory, chiasmata do not break, but their frequency may be reduced by their accumulation and cancellation at the ends of the chromosomes following terminalization. As a rule, terminal affinity prevents the chiasmata from passing off the ends of the bivalents during the prophase stages (DARLINGTON 1932).

On the classical theory, proximal interlocking should be much more frequent than distal interlocking. The reduction and terminalization of chiasmata shown in the interlocked bivalents of diagram 2 would result in the configuration shown in diagram 4. Distal interlocking, as shown in diagram 5, could occur only if chromosome B were locked with internode 4 of diagram 2 followed by a break in one of the chiasmata between internodes 2 and 4. If no breaks occurred in these chiasmata, they would presumably be cancelled by the opening-out of the proximal loop and by the

limitation in terminalization of the chiasma at the left of internode 4, caused by interlocking, so that at metaphase the interlocking would involve only proximal internodes. But if one of the two chiasmata breaks, then no cancellation would occur; and the distal interlocking would persist until metaphase (diagram 5) unless the terminal chiasma passed off the bivalent, releasing the locked bivalent B. The fact that terminal chiasmata are by far the most frequent in Tradescantia, Campanula and Oenothera would seem to indicate that terminal affinity does prevent chiasmata from passing off the ends of bivalents in these genera.

If the partial chiasmatypy theory of chiasma formation is correct, and if interlocking is at random, distal interlocking should be at least as frequent as proximal interlocking. And if the chiasmata are simply accumulated at the ends of the bivalents, as DARLINGTON (1932) suggests, there should be frequent distal interlocking of the type shown in diagram 6. Cancellation of some chiasmata would be possible so that distal interlocking at diplotene would appear as proximal interlocking at metaphase, but a single chiasma between the proximal loop and the distal loop involved in interlocking could not be cancelled, and many of the double chiasmata between the spindle fiber and a locking internode could not be cancelled, if crossing over is at random. We should, therefore, expect distal interlocking of the type shown in diagrams 5 and 6 to be more frequent than proximal interlocking, if the partial chiasmatypy theory is correct and if interlocking occurs at random.

TYPES OF CHROMOSOME INTERLOCKING IN OENOTHERA AND CAMPANULA

Interlocking of bivalent chromosomes has been described in Oenothera by CLELAND (1922) and others, and has been studied critically by CATCHESIDE (1931). *Oenothera Lamarckiana* mut. *pallescens* has a ring of 6 chromosomes and 4 bivalents as the typical meiotic configuration. In 292 nuclei CATCHESIDE found 465 bivalents locked with the ring of 6 and 111 bivalents locked *inter se*. Considering the ring of 6 as 3 potential bivalents, the total amount of interlocking is about 28 percent. Almost all interlocking in this species is proximal. Distal interlocking of the type shown in diagram 6 is described, but these cases are rare and "have been seen only three times in 331 nuclei at diakinesis." If these observations are representative, proximal interlocking is more than 200 times as frequent as distal interlocking in Oenothera. According to CATCHESIDE, the most frequent number of chiasmata seen in the bivalents at diplotene is three, though two or four and as many as five are occasionally found. The chiasma frequency per bivalent is 2.0 at metaphase. If the partial chiasmatypy theory of chiasma formation is correct, the percentage of distal

interlocking should be much higher than that observed if interlocking is at random and if chiasmata do not pass off the ends of the bivalents.

Interlocking has been observed in Campanula by GAIRDNER and DARLINGTON (1931). Proximal interlocking was found in about 20 percent of the nuclei in homozygous forms. The frequency of distal interlocking is not given, but it "seems to be much rarer." From two to six chiasmata were found in each bivalent at diplotene, and the most frequent number was three, while the chiasma frequency found at metaphase was less than two per bivalent. In both Oenothera and Campanula the only types of distal interlocking described are those where terminalization has reduced the terminal internode so that it cannot be seen as such, and the two bivalents seem to be in intimate contact. These figures are interpreted as the type of interlocking shown in diagram 6. In both Oenothera and Campanula, distal interlocking is very rare.

INTERLOCKING OF CHROMOSOMES IN TRADESCANTIA

Interlocking of non-homologous chromosomes has been found at meiosis in all diploid species of Tradescantia investigated. In a segmental interchange plant of *T. edwardsiana* the bivalents were locked with the chain or ring of four chromosomes in about 10 percent of the cells, while interlocked bivalents were found in only five percent of the cells (SAX and ANDERSON 1933). In regular diploids the percentage of interlocked bivalents varies greatly in the same species, and there is considerable variation in the same plant. The percentage of interlocking found, ranges from 1 percent to 27 percent. The percentage of interlocking for different plants of various species and the average chiasma frequency per bivalent is shown in table 1. The data are from counts made by EDWARD KING and in most cases were based on 100 pollen mother cells from each plant. The species used include *T. gigantea* Rose, *T. edwardsiana* Tharp, *T. bracteata* Small, *T. hirsutiflora* Bush, a variety of *T. reflexa* Raf., and natural hybrids between *T. reflexa* and *T. humilis* Rose.

In all the above plants described there were usually six bivalent chromosomes at meiosis. Occasional univalents were observed at metaphase, and in rare cases two bivalents were apparently connected at, or near, the fiber constriction points. No segmental interchange rings or chains were found, although they occur frequently in a few plants of some of the same species used (SAX and ANDERSON 1933).

The correlation between chiasma frequency and the percentage of interlocking is positive ($r = .4 \pm .1$). Interlocking might be expected to decrease the chiasma frequency by preventing the normal rate of pairing, and if the chiasma frequency is greatly reduced, interlocking would necessarily be reduced. But even in plants with the lower average chiasma frequencies,

most of the meiotic chromosomes are ring bivalents, since triple chiasmata are rare. And since almost all interlocking is proximal, the lower chiasma frequencies are not low enough to account for the lower percentage of interlocking. Interlocking does not seem to decrease chiasma frequency in Tradescantia, and the positive correlation between chiasma frequency and percentage of interlocking may be significant.

TABLE 1

Average chiasma frequency per bivalent and percentage of interlocked bivalents in 28 Tradescantia plants.

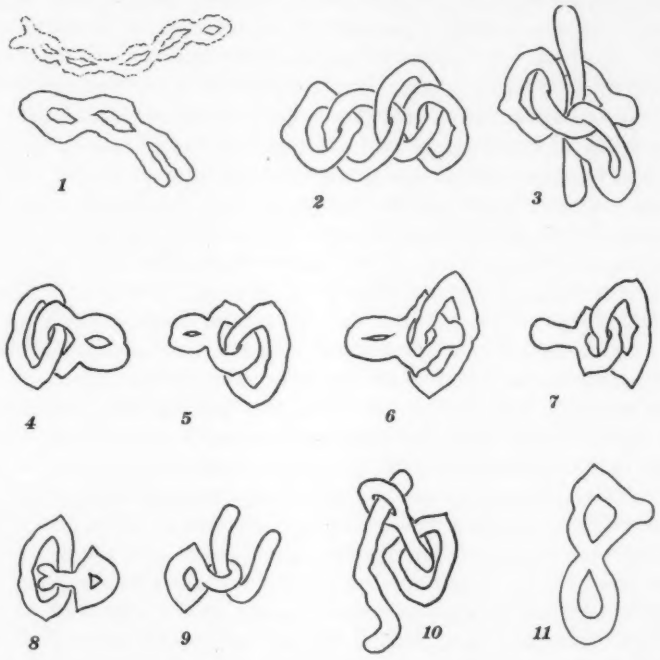
		Chiasma frequency per bivalent												
		1.6 1.7	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7 2.8	n
Percentage Interlocked Bivalents	0 5		2	2	2	2		1						9
	5 10				2	2	1	1			2		1	9
	10 15	1	1		1		1	1	1					6
	15 20								1		1		1	3
	20 25													
	25 30										1			1
		1	3	2	5	4	2	3	2		4		2	28

Tradescantia edwardsiana seemed best suited for a detailed study of the types of interlocking because the chromosomes were less contracted at metaphase, and the spindle fiber attachment points could usually be observed. Smears of pollen mother cells were made in a modified aceto-carmine solution containing about 3 parts of aceto-carmine, 1 part of glycerine, and enough iron alum solution to intensify the stain. The smears were heated and the cover glass pressed gently with absorbant paper to remove the excess fixing fluid and to flatten the cells. Such preparations, when sealed, remain in good condition for several months or longer.

About 9 percent of the bivalents were found interlocked in *T. edwardsiana*, plant number 15. In most cases the interlocking involved two ring bivalents. Photographs of these simple types of interlocking have been shown in an earlier paper (SAX and ANDERSON 1933). More complicated cases of interlocking are found frequently. Interlocking of four ring bivalents is shown in figure 2. Occasionally a ring bivalent is interlocked with two or more rings or rods (figure 3).

Bivalents with two internodes constituted about 4 percent of the meiotic

chromosomes. Of these double looped chromosomes about 10 percent were interlocked with a ring or rod bivalent. In these cases interlocking may be either proximal or distal. Types of proximal interlocking, where the ring or rod bivalent is locked in the internode containing the spindle fiber attachment point, are shown in figures 4, 5, 6, and 7. Types of distal interlocking with the ring or rod locked in the distal loop, are shown in figures 8 and 9. A total of 20 cases of proximal interlocking was found in these double internode chromosomes, while only 3 cases of distal interlocking



Drawings from aceto-carmine preparations of chromosomes of *Tradescantia edwardsiana*. ×1300.

FIGURE 1.—Two chromosomes from the same pollen mother cell. Three or four chiasmata per bivalent are often found at diplotene, and as many as five or six may occur.

FIGURE 2.—Interlocking of four ring bivalents at metaphase.

FIGURE 3.—Two ring and one rod bivalent locked in a ring bivalent.

FIGURES 4–7.—Types of proximal interlocking involving one bivalent with two internodes. Most of the interlocking found was between simple ring bivalents.

FIGURES 8–9.—Types of distal interlocking. Only 0.6 percent of all interlocking is distal.

FIGURE 10.—Proximal and distal interlocking in the same bivalent.

FIGURE 11.—An association of two bivalents attached near the fiber constriction points. These rare types of association may be the immediate result of segmental interchange following interlocking.

were found. In addition, one case of proximal-distal interlocking was found with a bivalent locked in each internode of the double loop chromosome (figure 10). In these chromosomes with two internodes the proportion of proximal to distal interlocking was 21:4, or 16 percent of the interlocking was distal. But for all interlocking, including the interlocked ring and rod bivalents, distal interlocking was found in only 0.6 percent of the cases, about the same percentage as CATCHESIDE observed in Oenothera.

In Campanula and Oenothera the apparent intimate contact of chromosomes at the terminal chiasmata is interpreted by GAIRDNER and DARLINGTON and by CATCHESIDE as distal interlocking of the type shown in diagram 6. No such types of interlocking have been observed in Tradescantia, although the chromosomes are undoubtedly more favorable for a study of interlocking than are those of Oenothera. Occasionally ring bivalents were found which seemed to be attached at or near the spindle fiber attachment points as shown in figure 11. In rare cases these chromosomes seemed to be in the form of a figure 8. They are not like the segmental interchange rings of four chromosomes, but they may be the immediate result of segmental interchange (SAX and ANDERSON 1933).

Interlocking in bivalents with three chiasmata occurs in the same proportion as interlocking in bivalents with two chiasmata. Nine percent of the rings were interlocked, while 10 percent of double internode chromosomes showed either proximal or distal interlocking. These proportions seem to indicate that the chiasmata do not pass off the ends of the chromosomes, thereby releasing distally interlocked bivalents.

The chiasma frequency at early diplotene could not be determined accurately. The prophase stages do not fix well, and chromosome development between pachytene and diakinesis seems to be very rapid. As many as five or six chiasmata per bivalent have been observed at diplotene, and bivalents with three or four chiasmata are often found. Two bivalent chromosomes from one pollen mother cell are represented in figure 1. One bivalent appears to have six chiasmata. The other bivalent has only three chiasmata, but it is undoubtedly further developed, and may not be typical for the chiasma frequency at early diplotene. The chiasma frequency at metaphase in *T. edwardsiana* No. 15 is 1.7 per bivalent. Apparently there is considerable reduction in chiasma frequency between diplotene and metaphase.

The chiasma frequency at diplotene in Tradescantia seems to be at least as high as it is in Campanula and Oenothera, where three per bivalent seems to be the usual number, and as many as five are found. With such a high chiasma frequency, distal interlocking should be at least as frequent as proximal interlocking if all internodes at diplotene are reductional,

as postulated by JANSSEN's hypothesis, and if interlocking occurs at random at any locus. But if the classical theory of chiasma formation is correct, distal interlocking can occur only in bivalents with three or more chiasmata on the same side of the spindle fiber attachment point. Since three of the chromosomes of *Tradescantia edwardsiana* are approximately isobrachial and the other three have submedian fiber attachment points, no distal interlocking would be expected unless four or more chiasmata were formed in a bivalent at diplotene. And if distal interlocking did occur in such chromosomes, cancellation of chiasmata would change some of the distal interlocking at diplotene to proximal interlocking at metaphase, the frequency depending on the amount of crossing over. If the classical theory of chiasma formation is correct, we should expect distal interlocking to be comparatively rare. In Tradescantia 99.4 percent of all interlocking is proximal, and only 0.6 percent is distal. Essentially the same proportion of proximal and distal interlocking is reported in Oenothera by CATCHESIDE. The relative frequency of the two types of interlocking in Tradescantia, Oenothera and Campanula seems to support the classical theory of chiasma formation.

The results cannot be taken as conclusive evidence in favor of the classical theory because the nature of the interlocking at pachytene is not known, and it is improbable that it can be accurately determined. It is possible that the interlocking usually occurs near the spindle fiber attachment point at pachytene. If the chromosomes begin pairing at the ends, any interlocked chromosome might be forced away from the ends and finally become locked near the middle of the chromosome. It is also probable that interlocking is associated with segmental interchange, since interlocking has been found only in genera where segmental interchange is commonly found. Even though no segmental interchange rings and chains are found in any of the Tradescantia plants described in this paper, it is possible that segmental interchange has occurred, involving chromosome segments too short to permit the formation of interchange rings, but perhaps long enough to cause some entangling of chromosomes at the time of pairing. If these short interchange segments are most frequent near the fiber constriction point, they might have some influence in causing an excess of proximal interlocking by the attraction of homologous segments in different bivalents, or by limiting chiasma formation to the distal regions of the bivalents.

SUMMARY

Interlocking of non-homologous chromosomes at meiosis has been found in all the diploid species of Tradescantia so far examined. In 28 plants studied critically, 8.7 percent of all bivalents were interlocked at meta-

phase. As has been reported for Oenothera and Campanula, distal interlocking was much less frequent than proximal interlocking.

If interlocking is at random at all loci of the pachytene chromosomes, distal interlocking should be at least as frequent as proximal interlocking if the partial chiasmatypy hypothesis of chiasma formation is correct, while distal interlocking should be very rare if the classical theory is correct. The rare occurrence of distal interlocking would seem to favor the classical theory. It is possible, however, that interlocking is not at random at pachytene, and that most interlocking occurs near the spindle fiber attachment point. It is also possible that distal interlocking is often released by the passage of a chiasma off the end of the bivalent, although the frequency of terminal chiasmata and the equal proportions of interlocking in single ring and double ring bivalents would not seem to support this suggestion.

Chromosome interlocking does not decrease the chiasma frequency per bivalent, and there may be some positive correlation between chiasma frequency and interlocking. The occurrence of interlocked bivalents is probably correlated with segmental interchange between non-homologous chromosomes, even though no multivalent associations are found at metaphase.

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A NOVEL TYPE OF HYBRIDITY IN FRAGARIA

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INTRODUCTION

In 1930 I described briefly a case of hybridity in which apparently there had been a union between an egg cell of *Fragaria vesca* L. ($n=7$) from Hawaii and a single genom ($n=7$) of *Fragaria virginiana* Duchesne ($n=28$) from the eastern part of the United States. The variety of *F. vesca* used as the female parent had white fruit. This plant was isolated in a room containing no other plants and was pollinated with pollen from a plant of *F. virginiana* having red fruit. Several hybrids were obtained having 35 chromosomes. They resembled *F. virginiana*. One plant only was found which had 14 chromosomes. Any strawberry specialist would have said that it was a plant of *F. vesca*, for it had all the major characteristics of this species. (In my preliminary paper, I stated that this plant resembled *F. vesca* in every respect, and then contradicted myself by saying that it showed "several other characteristics of *F. virginiana*." The phrase was "minor characteristics" in the manuscript, but the word "minor" was left out by mistake. I had not, at that time, noted the margin difference in the middle leaflet.) The differences noted were red fruits (not a specific distinction), rootstocks slightly stouter, and leaflets slightly firmer and darker in color (as in *F. virginiana*) but not beyond the limits of variation of other varieties of *F. vesca*. It was also found that the center leaflet was dentate only halfway to the petiolule—as is characteristic of the variety of *F. virginiana* used—instead of being dentate for three-fourths of the distance, as in the maternal variety.

The phenomenon was interpreted as above. It was thought to be similar to the "maternal," but red-fruited, type discovered by MILLARDET (1894) after an attempt to cross a white-fruited *F. vesca* with pollen from a red-fruited *F. chiloensis*. Later, it occurred to me that since the plant of *F. virginiana* which furnished the pollen had not been isolated, as was the mother plant, and since there were a few red-fruited plants of *F. vesca* in flower in another part of the greenhouse, there was a remote possibility that the plant in question was the result of crossing two varieties of *F. vesca*.

During the past four years, the original plant has been studied carefully, together with numerous clones. In addition, the behavior of 18 F_2 plants has been investigated. From the evidence thus obtained, there is little room to doubt the correctness of the original interpretation.

EXPERIMENTAL WORK

Observations on the supposed hybrid and the parents

Among the various species of the genus Fragaria, even those with different chromosome numbers are difficult to classify. By experience one learns to distinguish the various clones of a given group; but the criteria used are largely quantitative rather than qualitative. No one who has worked with strawberries is unable to separate *F. vesca alba* from *F. virginiana* without looking at the color of the fruit; yet when the descriptions are set down in words, the distinguishing marks mean little to those who are not familiar with the material. At the same time, it should be noted that the descriptions of *F. vesca alba* and *F. virginiana*, as set down in table 1, exhibit differences that are really decisive individually, since in most cases they do not overlap.

TABLE 1

Characteristics of F. vesca alba, F. virginiana, and the supposed diploid hybrid.

CHARACTER	F. VESCA ALBA	SUPPOSED DIPLOID HYBRID	F. VIRGINIANA
Chromosome pairs	7	7	28
Rootstock	slender	slightly stouter	medium stout
Runners	long	long	medium
Runners	light red	light red	red
Hairs	profuse, spreading	profuse, spreading	shorter, less spreading
Petioles	long, slender	long, slender	shorter, medium stout
Leaflets	almost sessile	almost sessile	slightly stalked
Center leaflet	2/3 dentate	1/2 dentate	1/2 dentate
Leaflets	light green, soft	light green, stouter	green, stiff
Scapes	strong, long, erect	strong, long, erect	strong, medium pendulous
Flowers	herm., small, white	herm., small, white	herm., medium, white
Calyx lobes	spreading or reflexed	spreading or reflexed	constricted
Stamens	short	short	long
Pollen	92 percent good	63 percent good	89 percent good
Fruit	conical, white	conical, red	shorter, red
Fruit	subacid	subacid	acid
Fruit flesh	soft	soft	firm
Achenes	superficial	superficial	sunken

Populations were raised from selfed seed of each of the pure species. The individual plants showed very little variability.

About 50 plants were also raised from runners of the original hybrid. They were as much alike as if they had been cut out with a die. Nine blossoming periods were observed. The flowers that came in the spring showed an average of 63 percent of good pollen as observed in aceto-carmine smears. Those which appeared in the winter had an average of 51 percent of good pollen. These figures should be compared with 92 per-

cent and 87 percent, respectively, for *F. vesca alba* and 89 percent and 84 percent, respectively, for *F. virginiana*. The ovules of the supposed hybrid all appeared to be functional, since berries perfectly filled with seeds were obtained. But the hybrid had one distinguishing feature apart from a marked heterosis and the points already mentioned. ***The anthers did not dehisce except rarely.*** Left to themselves the plants seemed to be almost completely sterile. When the anthers were scraped with a fine scalpel and the flowers were pollinated by hand, however, good fruits were obtained.

To sum up, then, the supposed hybrid was like the maternal parent, *F. vesca alba*, in all characters, except that it had red fruits, less extended leaflet dentation—particularly in the center leaflet—and somewhat stouter rootstocks and runners. It showed marked heterosis, had a much greater percentage of pollen abortion than either parent, and was almost lacking in the power to dehisce.

Observations on the F_2 generation

Ordinarily, selfed seeds of diploid varieties of Fragaria have germinated in percentages varying between 70 and 85. Three populations have been recorded in our experience where the percentage of seed germination was less than 60. The lowest percent was 43. ***In the case of the supposed hybrid, two tests gave the figures 15 percent and 11 percent, respectively.***

Eighteen plants have been raised to maturity and have been observed continuously for over three years since that time. This duration of time included 6 blossoming periods for some of the plants. This was true of No. 8 and No. 11, both being fertile. In addition, Nos. 3, 4, 5, 7, 8, 11, and 18 blossomed from 1 to 4 times and showed a reasonable degree of fertility by producing moderate quantities of fruit. The pollen, under aceto-carmine, varied in normality from flower to flower and even from anther to anther, as well as from plant to plant. The limits of variation were 39 percent and 79 percent. Dehiscence was fair, but not wholly normal. There were 5 red-fruited and 2 white-fruited plants (Nos. 7 and 8).

Plants 2, 16, and 17 did not flower during the three years that they were under observation as mature individuals. This is something that had never happened before in our experience with diploid Fragarias, though it had been comparatively common in the dwarf hybrids arising from the union of plants having different chromosome numbers.

Plants 1, 9, 10, 12, and 13, which blossomed once, and plants 6, 13, and 14 which blossomed twice, were completely sterile. Under aceto-carmine examinations, from 5 percent to 30 percent of the pollen of these plants appeared to be viable; but even with hand pollination no fruits were obtained. Only rarely did an anther dehisce.

These eighteen plants exhibited an amount of variation that was far beyond anything that we had seen among diploid Fragarias. No. 13 was

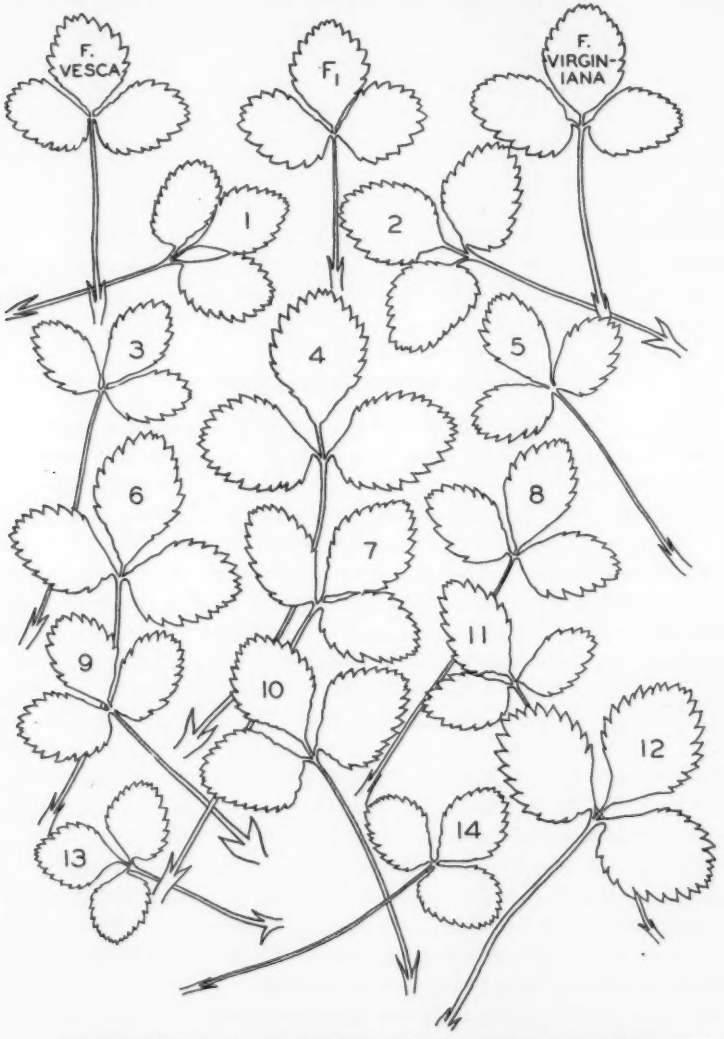

FIGURE 1.—Representative leaves of *Fragaria vesca alba*, *Fragaria virginiana*, the 14-chromosome F_1 plant, and plants 1–14 of the F_2 generation.

a dwarf, extremely compact; Nos. 1, 5, and 14 were very compact; Nos. 12 and 18 were very loose and straggly in habit; the remainder varied in

size, but were within the limits of variation of the diploid type of Fragaria.

Plants 12 and 15 (sterile) had twenty-one chromosomes, the remainder had fourteen chromosomes. These counts were made by my technical assistant, Miss ESTELLA HUMPHREY, from root-tip preparations. I had the opportunity of studying PMC slides from only two plants—both being of the diploid type. In both there was much more irregularity than I have seen in similar preparations of pure diploids made by Mr. ICHIJIMA and by Dr. YARNELL in this laboratory.

The variability of the plants in other decisive characters was as follows.

In the type of rootstock developed, 10 were slender, as in *F. vesca*, 4 were intermediate, and 4 were stout, as in *F. virginiana*.

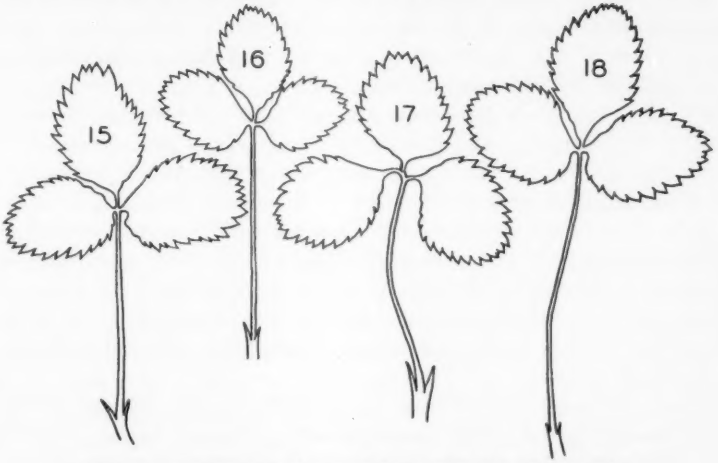

FIGURE 2.—Representative leaves of plants 15–18 of the F_2 generation.

In 10 plants the runners were as slender as those of *F. vesca;* in 5 plants they were as stout as those of the *F. virginiana* used; while in 3 plants they were much stouter than those of the *F. virginiana* used. There were 11 plants with long, 6 plants with medium, and 1 plant with short runners. And there was segregation in the two cases. The slender runners were not always long. In fact, in the plant where the runners were very short, they were also very slender. In 12 instances the runners were red or dark red, as in *F. virginiana;* while in 6 cases they were very light red, as in *F. vesca*.

The shape of the leaves, the dentation, the length of the dentate portion, the size of the stipules, the length of the petioles, and the length of the petiolules of each plant of the F_2 generation, together with the same characteristics for the parents and for the F_1 plant are shown in figures 1 and 2. We have found that the length of the petiolules and the length of

the dentate portion of the center leaflet form very critical diagnostic characters. The variation shown in the figures is much greater than that found in populations of either parental type. The leaf drawn is as near an average for the plant as one could determine; but of course a consideration of each plant as a whole gives a better idea of the amount of variation existing. Two important leaf characteristics not shown by the drawings are color and stiffness. There is a high degree of correlation between the two. In 10 plants the leaves were soft and light green; in 4 plants the leaves were dark green, tending to glossiness, and stiff. In only 1 diploid, No. 11, were the leaves dark green yet soft. The leaves of the triploids were dark green, very stiff and rugose.

In *F. vesca*, the scapes are long and erect; in the *F. virginiana* used, they are short and drooping. Of the fifteen F_2 plants which blossomed, 3 had long erect scapes, 2 had long drooping scapes, 5 had short scapes similar to *F. virginiana*, and 5 had very short scapes.

The flowers of each plant were hermaphroditic, 5 being much smaller than those of *F. vesca*, and 9 being about the size of the flowers of that species. Only 1 plant had flowers approaching the size of the putative male parent. All the plants but one had small concave petals as in *F. vesca*.

It is characteristic of *F. vesca* to have a spreading calyx in which the lobes are reflexed. In *F. virginiana*, on the other hand, the calyx is usually constricted, often closing around the base of the maturing fruit. This character was difficult to score on the F_2 plants by reason of the great amount of sterility shown, but there were only 2 plants that showed a tendency toward a constricted calyx.

One of the best diagnostic characters is stamen length. They are short in *F. vesca* and long in *F. virginiana*. Among the F_2 plants there were 10 with short stamens, 1 with stamens of medium length, and 4 with long stamens. In 5 plants the stamens were almost sessile—an extreme variation that I had not seen before.

The 7 plants that fruited (5 red, 2 white) had berries which were conical with long necks like those of *F. vesca*. The achenes were set superficially, except in one instance. There they were pitted as in *F. virginiana*. The flavor of all the fruits was subacid, and the flesh was mealy.

DISCUSSION AND SUMMARY

Let us recapitulate the more important facts and arguments involved in this study.

An isolated plant of *F. vesca alba* ($n=7$) was pollinated with pollen from a plant of *F. virginiana* ($n=28$) from the eastern United States. Several normal hybrids with 35 chromosomes were obtained. In addition there was a plant having 14 chromosomes that resembled the maternal parent

except as follows: noticeable heterosis, markedly lower pollen fertility, non-dehiscing anthers, red fruits, smaller amount of dentation on center leaflet, and stouter rootstocks and runners (the last two characteristics possibly due to heterosis and, at all events, unimportant).

The possible explanations of the origin of this plant are: (1) mutation; (2) contamination of the pollen of *F. virginiana* with the pollen of a red-fruited *F. vesca*, and (3) fertilization of the *F. vesca* egg cell having 7 chromosomes with a 7-chromosome genom from *F. virginiana*—the remaining 21 chromosomes being extruded into the cytoplasm and lost.

Obviously, the facts presented do not admit of the mutation interpretation. Moreover, there is no known instance of a white-flowered variety's giving rise to a red-fruit (dominant) mutation, although this fact does not preclude the possibility of such a mutation. We have to decide between interpretations two and three.

The characteristics of the F_1 plants (asexual reproduction) do not force a decision, though the non-dehiscing anthers and the greatly increased percentage of sterile pollen make one inclined to accept interpretation three. I say this in spite of the fact that a study of meiosis in this material gave us no clue as to the cause of the increased sterility. The P. M. C.'s appeared to go through their divisions normally.

The behavior of the eighteen F_2 plants, and the nature and extent of the variability which they exhibited, however, do force us, I think, to accept interpretation three. From previous experience, we know that plants of *F. vesca alba* crossed with pollen of the two similar red-fruited varieties that were in blossom at another part of the greenhouse when the flowers were taken from *F. virginiana* to make the cross under discussion, always flower, never show increased pollen sterility, and exhibit only a minor amount of variability. The variability found is that characteristic of an increased or decreased food supply. It affects the diagnostic characters used by taxonomists (noted in the text) so slightly that only a student of the genus Fragaria would notice the changes. And in no case do several diagnostic characters pass the limit of specific variability at one time.

In this small F_2 population of eighteen plants, it should be noticed, all the above phenomena occur. There are two triploids; three plants go through three years of growth after maturity without blossoming; eight plants are sterile; and variability exceeds specific limits in such diagnostic characters as the length of the petiolules, dentation of the center leaflet, length of the scape, size of the flower, and length of the stamens, besides showing an extraordinary variability in characters that have less specific constancy.

One may ask how such an odd case of hybridity could arise. On this

point a study of the maturation divisions in the pollen mother cells of some true hybrids between *F. vesca* and *F. virginiana* has yielded some information. In these thirty-five chromosome hybrids seven pairs are nearly always found, though there is sometimes a little irregularity. The remaining twenty-one chromosomes are commonly thrown out into the cytoplasm. Apparently the seven-chromosome gametes, which presumably are sometimes formed, are not functional—possibly because of certain imaginable cytoplasmic restrictions on functionability. But there seems to be no good reason why the fertilized egg cell in such a case should not eliminate the excess of chromatin and go through the divisions of ontogeny as a fourteen-chromosome plant.

If this interpretation is accepted for the novel hybrid described here, the phenomenon forms the basis for some very interesting reflections. The Fragarias are widely distributed. They exist in the wild as diploids, hexaploids, and octoploids. The limits of variation in the genus are narrow. The diploids are so similar that the experts do not agree on their classification. The hexaploids and octoploids are hardly more differentiated from the diploids than are the polyploids which have arisen from diploids in the genetic laboratory. That is to say, the octoploid Fragarias are as similar to *F. vesca* and its relatives as the tetraploids of Oenothera and Primula are to the diploids which gave rise to them. Fragaria is, then, a very constant genus, and one which might be expected to show a high degree of genetic compatibility among its members. Here there is a Hawaiian diploid so similar in gene constitution to an American octoploid that a genom from the latter will fit fairly harmoniously into the business of development when paired with a genom from the former species. Even meiosis in the hybrid exhibits no marked irregularity. At the same time, it should be noted that in the segregation which occurs in the F_1 generation, gene combinations are formed which do not fit so well. The result is plants which do not blossom and plants which are sterile when they do blossom.

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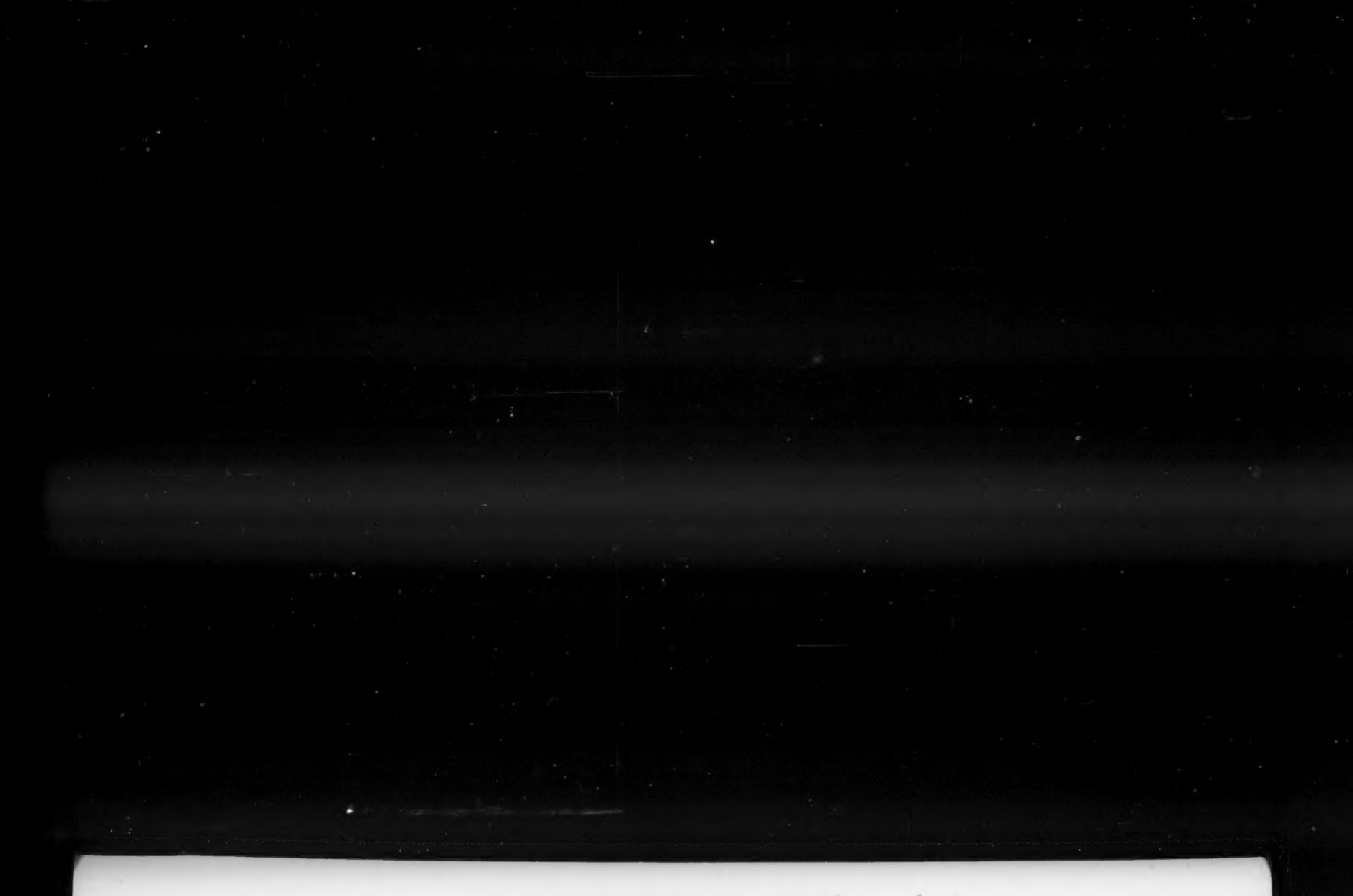
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